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FACTORS AFFECTING THE TESTS OF KIDNEY FUNCTION

RAYMOND C. HERRIN

Department of Physiology, University of Wisconsin Medical School, Madison

Tests for renal function as generally used are of three types. These are: the clearance method which includes the renal clearance of any substance which is in the blood and is excreted in the urine; the rate of excretion of some substance which is administered, such as phenolsulphonephthalein; and the determination of the maximum and minimum specific gravity of urine. This paper is a discussion of the various factors which may modify the first and last mentioned tests of renal function, almost exclusively from the viewpoint of the physiology of the kidney.

UREA CLEARANCE. *Nervous effects.* The question of nervous control or modification of urea clearance has stimulated many experimental studies which have been cited by Rhoads and co-workers (1) and by Smith (2). The latter has pointed out that anesthetics complicated the studies of most workers. Rhoads and associates found that novocainization or total section of the nerves in the renal pedicle of dogs with a kidney explanted under the skin had no effect upon urea clearance. Inasmuch as the dogs had been unilaterally nephrectomized renal hypertrophy probably had occurred. This could hardly be the cause of their negative results because the clearance could be greatly changed by means of diet. Page and Heuer (3) have reported that denervation in one patient with essential hypertension did not change the urea clearance. Freyberg and Peet (4) have reported the effect of bilateral splanchnicectomy upon urea clearance, maximum specific gravity and blood pressure in a series of 48 cases of hypertension. They considered that the hypertension was greatly relieved in some, but that there was no effect in others. In general they thought the changes in the kidneys were associated with the changes in blood pressure. In those patients who had a significant and maintained decrease in blood pressure, urinary abnormalities decreased or disappeared and the renal

function, if it had been impaired, improved. When hypertension was lowered in patients having normal renal function, the efficiency of the kidneys remained normal. When the hypertension was not favorably influenced, renal function remained unchanged or gradually became worse. Inspection of the data presented indicated to the writer that the operation resulted in 24 of 44 cases in no material change in urea clearance, in 9 the clearance decreased and in 11 there was a significant increase. In the latter group systolic pressure pre-operatively ranged around 225 to 260 mm. Hg and was markedly lowered by the operation.

The concentration of blood urea which might be expected to modify urea clearance was found by Addis and Drury (5) to have no effect upon the Addis urea ratio. This value when multiplied by 0.6 is that of urea clearance. In human subjects the blood urea changed over five-fold and in rabbits it was elevated from 27 to 1305 mgm. per cent with no change in the urea ratio. Möller, McIntosh and Van Slyke (6) reported that the administration of urea to normal men was without effect upon urea clearance. Van Slyke, Rhoads, Hiller and Alving (7) reported that in dogs with explanted kidneys the urea clearance and the proportion of urea removed from the blood by the kidneys was independent of the concentration of blood urea. At variance with these results are two recent reports. Fowweather (8) reported that after the administration of 15 grams of urea to each of 50 medical students who had no history or evidence of renal disease, the urea clearance data fell into a narrower range and were within the normal range, whereas before urea ingestion the results varied widely and many clearances were unexplainably below normal. However, the results of similar studies upon 20 patients, some of whom had nephritis, did not support Fowweather's claim. Morton and Mussey (9) reported confirmation of this observation on 50 normal individuals. The abnormally low clearance disappeared after ingestion of urea. In 15 cases of nephritis these workers considered that in mild nephritis the clearance increased and with more severe nephritis decreased after the ingestion of urea. Their data upon the nephritics seem too variable to permit generalization. The explanation for these two opposing results of urea ingestion is not evident. The diuretic action of urea by increasing urine volume might have influenced the clearances obtained by the latter workers. They did not report the urine flow before and after urea ingestion.

Another constituent of the blood affecting urea clearance is hemoglobin. Brown (10) in a study of urea clearance in pregnancy reported one case in which an increase in hemoglobin from 25 per cent to 50 per

cent was accompanied by a rise in clearance from 18 per cent to 66 per cent of normal. Fouts and Helmer (11) extensively studied urea clearance in cases of pernicious anemia. They concluded that there may be a marked increase in the clearance of such patients after a remission induced by liver extract. This occurred in 17 of 50 cases. They considered that the patient, having the complicating factors usually associated with an increased requirement of orally administered liver extract, was likely to show a low value for urea clearance even after the red cell count had reached normal. Also, the patient with a low clearance was more likely to require liver extract by injection to maintain a normal red cell count than the patient with a normal clearance. Of course the relapse and remission of pernicious anemia may affect the kidney directly as well as by the level of hemoglobin.

Diet has a marked effect upon urea clearance especially in dogs. Addis and Drury (12) reported that the urea ratio was increased in the human subject 3 to 5 hours after ingestion of a mixed meal, glutamic acid or milk. Cane sugar or alcohol had no apparent effect. Jolliffe and Smith (13) found that in dogs the urea clearance was reduced under the conditions of fasting or a low protein diet, but was greatly increased by a meat diet. The effect of the meat feeding persisted 16 to 18 hours after the last meal. This effect of meat feeding in dogs was confirmed by Van Slyke and associates (14). Pitts (15) found that meat, casein, glycine and alanine increased urea clearance in dogs. However, the protein intake in the human subject has less effect upon urea clearance. Cope (16) reported that raising the protein content of the diet of hospital patients from 75 to 120 grams was without consistent effect upon urea clearance. On the other hand, lowering protein intake from 75 to 40 grams was accompanied by depression of the clearance in 11 of 12 subjects with normal or nearly normal renal function. In 3 patients whose clearance was below half the normal, the low protein diet had little effect. Goldring and associates (17) reported that the urea clearance at urine flows above the augmentation limit in normal men subsisting on diets containing 9 or 280 grams of protein per day, was reduced about 23 per cent at the lower protein level but showed no elevation at the higher protein level as compared with a control period when 100 grams of protein per day was given. Farr (18) found in 4 children, aged 4 years, with the nephrotic syndrome, that the urea clearance varied markedly with the protein intake. Protein intakes of 0.5, 1, 2, 3 and 4 grams per kilo per day were accompanied by average urea clearances of 73, 88, 178, 184 and 216 per cent of mean normal,

respectively. Administration, during low protein periods, of urea sufficient to produce urea outputs like those observed during high protein intake caused relatively slight increase in urea clearance.

The vitamin A intake has been demonstrated by Herrin and Nicholes (19) to have an effect upon the magnitude of urea clearance. Avitaminosis A in dogs resulted in urea clearances which were about 70 per cent of normal and administration of large doses of vitamin A to these deficient dogs and normal ones resulted after a few weeks in clearances markedly above normal. The variations in urea clearance were effected by parallel changes in the rate of glomerular filtration and were due to changes in function and not to anatomical changes in the kidney. The administration of large doses of vitamin A to normal human subjects receiving their usual mixed diet produced variable results. In 2 of 17 subjects the urea clearance did not change, in 14 the clearance increased 11 to 15 per cent and in 7 the clearance elevation ranged from 24 to 91 per cent. The subjects in the latter group seemed to be the type who easily gain in body weight (20).

Variations in the intake of NaCl from minimal to large amounts was found by Cope (16) to have no effect upon urea clearance in human subjects. In one instance as much as 65 grams were ingested and excreted in the urine at high concentration with no effect upon clearance. Landis, Elsom, Bott and Shiels (21) extensively reviewed the literature and studied the effect of NaCl restriction upon urea clearance in patients with renal insufficiency. They used 12 hour or 24 hour clearance periods because they found less variation in the data than that obtained with a one hour period. With the diet and fluid intake maintained constant, a restricted intake of NaCl was accompanied by a slight elevation of plasma urea and slight diminution in the 24 hour urea clearance. Administration of NaCl reversed these effects. In one patient with advanced renal insufficiency plasma urea decreased from 154 to 26 mgm. per cent during NaCl administration. Acute restriction of NaCl produced hypochloremia with characteristic symptoms, and temporary retention of urea, creatinine and phosphate. Administration of NaCl relieved symptoms and diminished the blood content of urea, creatinine and phosphate. In this patient urea clearance over 24 hours was slightly lower during and shortly after NaCl restriction, and was higher during NaCl administration. The effects of lack of NaCl appeared only after several days due, they thought, to the time required for depletion of body stores of Na.

Effect of age. The urea clearance of 9 normal infants, according to

Schoenthal, Lurie and Kelly (22), when corrected for surface area, agreed with values established by Van Slyke for adults. The average urea clearance in children was considered by Payne and Shukry (23) to be higher than in adults. In 39 children free of renal tract disease, the average was 80 to 140 per cent with only 8 cases below 80 per cent.

Cullen, Nelson and Holmes (24) determined urea clearance in 62 children with no evidence or history of renal disease. The data were analyzed biometrically. They concluded that the distribution and mean values of the urea clearances corresponded to those of normal adults. The ages ranged from 5 to 13 years and there was no significant difference in clearance with age or sex. Lewis and Alving (25) measured urea clearance in 100 normal men, 40 to 89 years of age, twenty in each decade and in 2 men 91 years and one man 101 years of age. The clearance declined with age. Their equation for the decline in the men aged 40 to 89 years is, urea clearance in per cent of clearance of normal young men, $= 136.6 - 0.912 \times$ age in years. The decline over this 50 year period was 46 per cent. The mean values in succeeding half-decades or decades suggested to these workers, that the decline does not follow a straight line regression, but an S-shaped curve, being arrested from 55 to 65 years and accelerated from 65 to 75 years. Furthermore, the blood urea in this same group over the 50 year period increased 46 per cent. The gradual increase of blood urea followed sometime after the decrease of urea clearance.

Muscular activity. Addis and Drury (5) found that the urea ratio decreased markedly in a human subject during an hour of strenuous exercise but was at a normal level during the first and second hours after the exercise. MacKay (26) noted a similar effect of exercise upon the urea ratio. Van Slyke, Alving and Rose (27) found that in normal subjects, or in nephritics with more than 50 per cent of normal blood urea clearance the clearance was not materially different, whether the subjects were kept in bed during the determination or were walking about. In 3 of 12 nephritics, with less than 50 per cent of normal urea clearance, the mean clearances observed with the subjects in bed, rising and walking about were 44, 66 and 67 per cent respectively of the clearance observed in the same patients during bed rest. Severe exercise taken by 3 subjects with normal urea clearance depressed the clearance somewhat but in only 3 out of 22 clearances determined during heavy exertion were the values below 70 per cent of the mean normal. Light and Warren (28) determined the effect of playing the regulation games of football, soccer and basketball by boys 14 to 20 years of age, upon

their urea clearance and the protein content of urine. A definite fall in clearance occurred in every one of the 29 boys during the period of the game. The clearances during the games ranged from 47.5 to 37.3 per cent of the subject's normal. There was no correlation between the decrease in clearance and degree of albuminuria.

Effect of pregnancy. Brown (10) in 1938 extensively surveyed the literature. The clearance in normal pregnancy was found to be markedly elevated by Cadden and McLane, Hurwitz and Ohler, and Nice but was decreased in the reports of Cantarow and Ricchiuti, and Dieckmann (29). In Brown's series of 41 cases of normal pregnancy, of 54 clearances, only 2 were below 100 per cent and these occurred in the same patient. The average urea clearance was 134 per cent with a range of 78 to 195. The clearance did not change with increase in the gestation period contrary to the decrease presented in an earlier report. Age and parity did not have any influence upon the urea clearance.

Brown concluded on the basis of 196 clearances upon 170 cases of toxemia of pregnancy that the urea clearance was of considerable value in the differential diagnosis between pre-eclamptic toxemia, chronic nephritis and essential hypertension. Before delivery a high value excludes chronic nephritis and if the blood pressure remains high with little other evidence of toxemia and the clearance is high, the case is probably one of essential hypertension. If the clearance is low the result is of no differential diagnostic value in pregnancy but the clearance after delivery is significant, in that most patients with pre-eclamptic toxemia show a rapid rise within a week or two of delivery while in chronic nephritis the percentage use is much less and the clearance never reaches a normal level. The follow-up of patients after delivery showed that if the clearance is high during the toxemia the prognosis is good even if the degree of toxemia is clinically severe. Chesley (30) has reported a study of renal function in 599 cases of toxemia and 119 of normal pregnancy. He concluded that the urea clearance is about the same in normal pregnancy as out of pregnancy, and that in eclampsia, pre-eclampsia and hypertension the urea clearance does not differ significantly from values observed in normal patients. This striking divergence of Chesley's conclusions of the effect of normal and abnormal pregnancy upon urea clearance from those in the literature is not comprehensible. He has quite justifiably criticised some of the reports of low clearances in the toxemias of pregnancy on the basis that the urine flows were extremely low. Generally the reports give no actual urine flows but most of the workers calculated either standard or maximum

urea clearances. The excellent paper of Nice presents conclusive evidence that in normal pregnancy the urea clearance is higher than when the same individual is non-pregnant. Inasmuch as she determined clearances from the fifth month of gestation to the eighth month postpartum, her data are far superior to a single urea clearance which is merely referred to a standard. This method of using the urea clearance is a control for the tremendous variation in urea clearance from one individual to another. For this same reason the report of Brown that the clearance in cases of toxemia rapidly rises after delivery is particularly significant.

Influence of hormones. The subcutaneous injection of adrenalin in the rabbit was found by Addis, Barnett and Shevky (31) to be followed by an increase in the urea ratio. With large doses a decrease occurred. The subcutaneous injection of pituitrin was followed in all effective amounts by a decrease in the urea ratio (32). Addis and Drury (12) reported that a large dose of adrenalin injected intravenously in a human subject greatly reduced the urea ratio. Pituitrin injected subcutaneously markedly reduced the ratio in 2 subjects. With both drugs the reduction in clearance was paralleled by a very great diminution in urine flow. Farr, Hare and Phillip (33) produced a persistent polyuria in cats by surgical intervention of the hypothalamico-hypophyseal complex. They found no significant difference between the urea clearance of normal cats and that of cats with diabetes insipidus. Pitressin acted on cats with this type of diabetes insipidus to reduce urine volume but increased their urea clearance as well as the clearance of normal cats. Anterior pituitary substance increased the urea clearance of both normal cats and those with diabetes insipidus. This effect in the latter animals was not solely due to increased urine volumes. White and Heinbecker (34) produced experimental diabetes insipidus in dogs by surgical interference with the hypothalmico-hypophyseal system. They found the creatinine clearance unchanged during the transitory phase of experimental diabetes insipidus and the urea clearance somewhat elevated probably because of the increased urine flow. Creatinine clearance in the dog measures glomerular filtration. Both clearances were normal during the normal interphase. Soon after onset of permanent polyuria both clearances gradually fell. Creatinine clearances reached a level about half of normal by 2 to 4 weeks after onset of permanent polyuria. Urea clearance fell less than the creatinine during permanent polyuria and in some cases did not fall at all. Under the influence of anterior lobe administration, creatinine clearance rose slightly, the urea clear-

ance to a greater extent so that the urea/creatinine clearance ratio rose to 0.8 or 0.9.

Pitts (15) reported that administration of thyroxin to a dog resulted in an elevation of urea clearance. The effect was not seen until the second day. Bartels (35) has reported the urea clearances of 23 clinical cases of hyperthyroidism. In 6 cases the clearances ranged between 65 and 75 per cent and all other values were within the normal range. He concluded that there was no relationship between urea clearance and the type of hyperthyroidism, duration of disease, weight loss or level of the basal metabolic rate. The finding of clearances within the normal range was not expected on the basis of Pitts' work. It may be that the normal clearances represent some adaptation on the part of the kidney as has been seen in the case of the effects of protein and vitamin A in the diet. This adaptation might have been revealed if clearances had been determined after surgical thyroidectomy.

Effect of pharmacological agents. Page (36) has reported that the diuretic, salyrgan, and the xanthine diuretics, caffein and diuretin, did not alter the urea clearance in hypertensive and nephritic patients. He (37) also found that the urea clearance in essential hypertension was not lowered when the blood pressure was lowered either spontaneously or by sulphocyanate or by intramuscular injection of colloidal sulphur. Fulton, Van Auken, Parsons and Davenport (38) have reported that the diuretics, salyrgan, novasurol, ammonium chloride, urea, digitan, theocin, theocin-sodium-acetate, theophylline-ethylene-diamine, theobromine-sodium salicylate and caffeine, had no consistent effects upon the maximum urea clearances in dogs. Payne and Shukry (23) reported that 10 to 30 minutes of ether or nitrous oxide anesthesia in children had no effect upon the urea clearance determined immediately after the anesthesia or 10 days later. Orth and Stutzman (39) have surveyed the literature of the effects of anesthetics upon kidney function. These workers subjected dogs to surgical anesthesia for 60 to 75 minutes with cyclopropane, ether or chloroform. Maximum urea clearances, determined after the urine flow had returned to normal, showed no significant deviations from the control values. This report emphasizes the well known oliguria which accompanies anesthesia and indicates that at the time of spontaneous recovery of urine flow the urea clearance is also normal. Smith (46) has very justly condemned experiments performed with anesthesia and surgical procedures as yielding little information on the normal physiology of the kidney.

Removal of one kidney in dogs was reported by Van Slyke and asso-

ciates (7) to be followed by an average increase of 43 per cent in urea clearance of the remaining kidney. The blood flow was increased 68 per cent and oxygen consumption 81 per cent. The maximum increase occurred about one month after the nephrectomy.

Effect of temperature and bodily infections. Goldring (40a) has cited the literature and reported that in the febrile stage of acute rheumatic infection, urea clearance values are usually higher than the highest observed normal and during the afebrile convalescent period the clearances are usually lower than the lowest observed normal. In 13 patients he (40b) found the urea clearance increased as a rule during the acute stage of penumonia and was slightly to moderately depressed during the convalescent stage. Page (41) has cited the contradictory literature regarding the observed effects of renal diathermy. He administered diathermy treatment for the period of an hour to 14 subjects that included normal subjects and patients with hemorrhagic Bright's disease, nephrosis and essential hypertension. He reported no significant change in urea clearance, diuresis or blood pressure. Grant and Medes (42) have reported an elevation of the creatinine clearance in normal, unanesthetized dogs whose body temperature had been elevated by conventional diathermy. It is believed that in this animal creatinine clearance approximates the volume of glomerular filtration. Farr and Moen (43) have reported that fever induced in rheumatic patients by a heating cabinet was associated with a decrease of 25 to 38 per cent in the urea clearance. In two cases oliguria occurred even though large quantities of water had been ingested. Nicholes and Boynton (44) have found that an increase in the body temperature of dogs, produced by conventional diathermy or electromagnetic induction, was associated either with no change in creatinine clearance or a marked reduction. The evidence points to the idea that increased body temperature either has no effect on urea clearance or lowers the clearance, perhaps because of a circulatory adjustment to the hyperthermia. Chasis, Ranges, Goldring and Smith (45) have reported that pyrexia produced in human subjects by typhoid vaccine or pyrogenic inulin preparations very greatly increased the inulin clearance which is a measure of the volume of glomerular filtration. Renal blood flow was likewise much increased. Also, Nicholes and Boynton obtained a marked increase in the creatinine clearance by injecting typhoid vaccine into the same dogs that had shown no increase in clearance during the experiments of electrical heating. Furthermore, Smith (46) obtained the same effect of the pyrogen upon the inulin clearance and renal blood flow after the rise in blood

pressure, the chill and nausea and hyperthermia had been eliminated by pre-medication with amidopyrine. These studies strongly suggest that the increase in urea clearance which has been reported to occur in infections is not due to the hyperthermia but rather to the metabolic changes responsible for the fever.

Miscellaneous. The urea clearance has been reported to be within the normal range in psychotics even though over 20 per cent of the hospital admissions had albuminuria (47).

Page and Alving (48) reported that the operations of tonsillectomy, adenoidectomy and tooth extraction had usually no effect upon urea clearance. In 3 of 31 cases, however, the urea clearance was depressed in the days immediately post-operative. The first two operations were performed under gas-oxygen-ether anesthesia and the extractions under procaine anesthesia. MacKay (26) found a diurnal variation in the urea clearance of normal subjects and patients with Bright's disease. The standard clearance was depressed during the first hour after rising, after which it increased from 9 to 12 a.m. In this period there was the minimal variation. After lunch hour the clearance dropped and rose again in the late afternoon and evening. The standard clearance was much lower during sleep.

Rate of filtration and degree of tubular absorption of urea. In the normal kidney the magnitude of urea clearance is determined by the rate of glomerular filtration and the degree of tubular reabsorption. In the dog the effect of dietary protein and vitamin A upon urea clearance was by way of a change in the rate of glomerular filtration. Those factors which alter urea clearance over a considerable period of time probably do so by changing the glomerular filtration rate, rather than the relative amount of urea absorbed by the tubules. Normally, 40 to 50 per cent of the filtered urea is reabsorbed by the tubules and there are certain peculiarities about this absorption. Addis and Van Slyke early appreciated that urea excretion is less at low urine flows and Van Slyke came to the idea that in man urea excretion, at urine flows below 2 cc/min., varied as the square root of the urine flow, whereas above this flow it was independent of urine flow. Chesley (49) has reported that the formula of Van Slyke used in calculating standard urea clearance gave erroneously low results when the urine flow fell below a critical volume which he decided was about 0.35 cc/min. in adults. Below that urine volume the U/B reversed its trend and decreased with further decrease in urine volume. In a later paper he proposed that in oliguria "minimal" urea clearances be calculated by a formula provided with a constant.

Van Slyke, Rhoads, Hiller and Alving (7) found that in dogs with one kidney and it explanted subcutaneously about 6 to 12 per cent of the urea in the arterial blood was extracted by the kidney as the blood makes one renal circuit. However, they observed several times that the renal venous blood would have more urea than arterial, as much as 27 per cent more in some instances. Gordon, Alving, Kretzschmar and Alpert (50) found that extraction of urea by the explanted kidney of dogs was extremely variable and that this was due to changes in the tubular absorption and not to changes in filtration. The absorption varied from none to all of the filtered urea and the average was between 40 and 50 per cent which average was usually approximated within any period of 30 to 60 minutes.

The urea clearance when determined on a rising urine flow is markedly higher than those values obtained with a corresponding urine flow but with the rate of flow constant or falling. This aspect has been discussed more completely elsewhere (2). It is not due to increased filtration since the urea clearance rises relative to the filtration rate. Recently Shannon (51) has studied urea excretion in the dog during forced diuresis. He reported that as the creatinine U/P ratio is reduced from 10, progressively less urea is reabsorbed. Inasmuch as he found that the urea/creatinine ratio could be extrapolated to 1.0 at a creatinine U/P ratio of 1.0 he considered this as evidence there is no active reabsorption of urea. He has explained the absorption of urea on the basis of the hypothesis that water reabsorption takes place at two sites in the nephron. The diffusion gradient created by the reabsorption of water in the proximal tubule accounted for the deficit in the urea clearance of the highest urine flow during water diuresis. The further deficit associated with low urine flows he attributed to diffusion in the distal portions of the nephron.

Methods of determining the rate of glomerular filtration. Convincing evidence for the idea that the inulin clearance measures the rate of glomerular filtration has been summarized by Smith (2). Richards, Bott and Westfall (52) have brought forth additional evidence that inulin is excreted solely through the glomerulus. Perfusion of inulin through the renal portal vessels of the frog's kidney did not result in the presence of inulin in the tubular lumen. In dogs and rabbits filtration in the kidney was temporarily abolished by lowering the renal blood pressure. The blood circulating through the kidney during this condition contained inulin and those substances, phenol red, diodrast and hippuran which have been shown to be secreted by the tubules. Although the urine subsequently obtained contained enough of the secre-

table substances to prove that secreting power of the tubules had not been abolished, it contained so little inulin as to warrant the conclusion that the tubules in dogs and rabbits are not capable of secreting inulin. Corcoran and Page (53) and Alving, Rubin and Miller (54) have developed methods of determining inulin colorimetrically, which method permits the analysis of samples containing very small amounts of inulin. Miller, Alving and Rubin (55) have reported the determination of inulin clearances in human subjects at very low plasma levels and found that these values closely approximated the clearances obtained at higher plasma concentrations. The results indicated to them that there was no tubular excretion or reabsorption of inulin.

Barnett (56) has developed an indirect method of estimating the inulin clearance and used it with infants and children. Inulin is injected intravenously and two samples of blood are drawn 2 and 3 hours later, the exact time after the injection being noted. On semi-logarithmic paper the inulin concentrations of the two blood samples are plotted on the logarithmic coördinate against time on the linear coördinate. The slope of a straight line connecting these two points is determined by the formula, slope $= \frac{K - \log C}{t}$, where K is a constant whose value is determined by extending the line to zero time and taking the log of the concentration at this point; C is the plasma inulin concentration in milligrams per cent at any given time, t, in minutes after the injection. The relation of the slopes of the lines obtained to inulin clearance as actually determined in children 5 to 15 years of age showed in Barnett's opinion a fairly good relationship between the two factors. He applied the method to 7 apparently normal full term newborns, 4 to 9 days of age, and found slopes corresponding to inulin clearances of 20 to 40 per cent of the normal, 120 cc. per minute. In 4 children, aged 14 days to 7½ weeks, the slope of the lines corresponded to inulin clearances of 50 to 90 per cent. In 2 children 6 and 10 years, the slopes corresponded to normal clearances. In each group, the clearance was corrected for body surface area. This method may be very useful but needs further study. This report indicates that in the very young glomerular filtration is impaired which would not be expected on the basis of urea clearance determinations (22).

Rehberg (57), the first investigator who attempted to measure glomerular filtration in the human used the clearance of exogenous creatinine as a measure of this process. Later it was demonstrated by Shannon (58) that exogenous creatinine is excreted in the human by both

glomeruli and tubules. Miller and Winkler (59) found that the clearance of endogenous creatinine in normal subjects was usually but not invariably equal to the inulin clearance. These workers also observed that in subjects with chronic nephritis and depressed renal function the endogenous creatinine clearance was usually higher than the inulin clearance. Another peculiar observation noted in this latter group of subjects was the failure of the exogenous creatinine clearance to rise regularly above the endogenous creatinine clearance after the injection of creatinine, as it does in the normal subject.

Other clearance methods which may be used to measure certain aspects of renal function. After the experimental demonstration by Elsom, Bott and associates (60) that diodrast and hippuran are excreted in large part by the renal tubules of the dog, rabbit and man, Smith and co-workers (61) have applied the diodrast clearance, because it is the maximum clearance known, as an indirect method of approximating the renal plasma flow. The theoretical principle of this method is that the kidney completely removes the diodrast from the plasma, so that if the concentration of diodrast in the arterial blood and the quantity of diodrast in a minute's excretion of urine are known, the calculation of renal plasma flow is simple. This method of determining renal plasma flow would be based on certain assumptions. One, that there was no storage of the diodrast in the tubular epithelium or any other cells of the kidney; second, that either the red cell membrane was completely impermeable to the diodrast or that there was free and rapid diffusion of diodrast across the red cell membrane, and third, that the plasma of the renal vein blood was free of diodrast.

Smith made certain experimental observations in normal human subjects which he interpreted as indicating a negligible store of diodrast within the kidney. Elevating the plasma concentration of diodrast depresses the ratio of phenol red clearance to inulin clearance and lowering the plasma diodrast permits increase in the ratio. The curves expressing the relationship between these two factors when obtained with rising and falling plasma concentrations of diodrast are not superimposable. However, when both curves are corrected for the delay time of excretion occasioned by the circuit substances must take from the site of injection to the collected urine sample, which time was experimentally determined by injecting phenol red intravenously and noting its initial appearance in the urine, the curves are superimposable except for a slight divergence at low plasma diodrast values. It would appear that the degree of sensitivity of the mechanism for excretion of phenol red to the presence

of diodrast is one uncertainty in Smith's interpretation. White (62) has reported that the diffusion equilibration rates of diodrast between cells and plasma in vivo were high enough in the dog so that the cell diodrast content of renal vein blood was lower than arterial cell diodrast. The blood cells therefore contribute diodrast to the urine. This behavior would give values for renal plasma flow that were too high. Furthermore, at arterial plasma concentrations of diodrast below that at which the clearance begins to be self-depressed, the extraction varied markedly and averaged 74 per cent. This failure of the kidney to free the plasma of diodrast completely in one passage of the blood would give values for renal plasma flow which would be too low. White, Findley and Edwards (63) found that the blood cells in the human contribute less diodrast to the urine than is the case with dog's blood. They also found that the diodrast plasma clearance begins to be self-depressed at lower plasma diodrast levels in the dog than in man which suggested to them that human kidneys extract diodrast more efficiently than do dog kidneys. These studies would indicate that in the dog renal plasma flow determined by the diodrast plasma clearance might be in error but in man the two errors are apparently smaller and since they are in opposite directions this method may closely approximate the renal plasma flow.

The physiology of the renal circulation has been quite recently reviewed by Smith (46). Certain features of this subject might be mentioned in this paper. Considerable experimental evidence indicates that the renal circulation possesses a great autonomy. The circulation through the kidney remains rather constant in spite of a changing arterial pressure. Also, this capacity of circulatory adjustment is not impaired by denervation. The renal circulation does not show the reactive hyperemia after a period of ischemia which is peculiar to other organs. It has been reported that the kidney vessels of the anesthetized dog do not participate in the reflex vasoconstriction produced by hemorrhage. It is also reported that the renal vessels in the dog are not directly affected by carotid sinus reflexes. Denervation does not produce hyperemia in the normal dog or man. After the renal artery is partly closed by a clamp, the renal blood flow returns spontaneously after some delay to the normal. The renal blood flow must be reduced about 40 per cent by the Goldblatt clamp in order to produce experimental hypertension in the dog. On the other hand, the oxygen arterial-venous difference approximates the normal (46). The experimental observations that anoxia in the frog results in constriction of

the glomerular arterioles may have some connection with this phenomenon. Adolph (64) directly observed that displacement of O_2 by nitrogen resulted in constriction of the glomerular vessels. At 22°C. only 4 per cent of an atmosphere of O_2 prevented this constriction and larger concentrations had no apparent effect on glomerular flow. Beck, Kempton and Richards (65) directly observed that when the perfusing fluid contained cyanide the glomerular arterioles constricted and other vessels in the kidney dilated, with the result that the renal blood flow was not severely curtailed. These experiments with the frog seem to suggest that in anoxia consequent to clamping of the renal artery, certain nephrons might be eliminated from the circulation and the others which receive blood extract O_2 at the usual rate.

The diodrast clearance in 9 normal women was found by Chesley and Chesley (66) to give an average renal plasma flow of 856 cc/min/1.73 $\bar{M}^2$. The range extended from 694 to 1233 cc. The plasma clearance of diodrast they found to be 18 per cent higher in the pregnant woman but they explained this by the hydremia of pregnancy. White, Findley and Edwards (63) obtained an average diodrast plasma clearance for 11 subjects of 497 cc/min/1.73 $\bar{M}^2$. Smith and associates (67) obtained a mean diodrast plasma clearance in 35 normal subjects of 669 cc/min/1.73 $\bar{M}^2$. The range extended from 455 to 921 cc.

Only 3 factors are known which produce a marked increase in renal blood flow. Unilateral nephrectomy in both dog and man result, in the course of a few months, in 70 to 100 per cent increase in the flow to the remaining kidney. In the unilateral nephrectomized dog a high meat diet increases renal blood flow to the explanted kidney. Typhoid vaccine or pyrogenic samples of inulin produce a marked increase in renal blood flow in man (46).

Simultaneous determination of renal plasma flow and the inulin clearance makes possible calculation of the filtration fraction or the extraction ratio of inulin, by which term is meant the per cent of the plasma inulin which is filtered at the glomerulus. Inulin clearance/renal plasma flow $\times$ 100 = filtration fraction. An increase or decrease in the filtration fraction is generally interpreted as an increase or decrease respectively, in the tone of the efferent arteriole. The renal hyperemia produced by a pyrogen is considered to be brought about by dilatation of the efferent arteriole inasmuch as the filtration fraction markedly falls. Adrenalin, orthostatic vasoconstriction and psychogenic vasoconstriction, all increase the tone of the efferent arteriole (46).

Inasmuch as the filtration rate can be determined by the inulin clear-

ance, certain functions of the renal tubules can be quantitatively measured. These possibilities have been pointed out by Smith (68). Shannon and Fisher (69) have demonstrated that when blood glucose is elevated sufficiently, the rate of reabsorption of glucose by the tubules in the dog becomes maximal and rather constant. This has been shown to hold for the human kidney and the maximal rate of glucose absorption can be determined and is designated glucose Tm. Glucose Tm would evaluate the number of normal active nephrons and the tubular absorptive mass of the kidney since the maximal quantity of glucose reabsorbed would require the nephron to have a functional glomerulus and tubule with this absorptive capacity. Elevation of plasma diodrast sufficient to exceed the excretory capacity of the tubules would give diodrast Tm or the maximal quantity of diodrast excreted by the tubules per minute. This value would serve to measure the mass of active excretory tubules, whether glomerular or aglomerular. Inulin clearance of course measures all functional glomeruli regardless of whether the tubule is physiologically functional or impotent. Goldring, Chasis, Ranges and Smith (67) found an average glucose Tm of 344 mgm./min. corrected to a surface area of $1.73/\bar{M}^2$ in 14 subjects with no evidence of renal disease. They have pointed out certain factors which might influence the accuracy of this determination. The rate of glomerular filtration and blood glucose must be very accurately determined since a small error greatly changes the amount of glucose filtered. The amount of glucose delivered to the tubules should exceed the maximal rate of glucose absorption by at least 20 per cent. Furthermore, the elevation of plasma glucose to the necessary 350 to 700 mgm. per cent probably causes expansion of plasma volume which might disturb the normal equilibrium in the kidney. This same group found the diodrast Tm in 35 subjects ranged from 36.6 to 72.0 with a mean of 51.6 mgm. of iodine per $1.73/\bar{M}^2$ per minute. White and associates (63) obtained a value of 44 mgm.I./min./1.73 $\bar{M}^2$ for diodrast Tm on 3 normal subjects.

Clearances in diseases of the kidneys. The changes in the urea clearance of 67 patients with Bright's disease, the hemorrhagic, sclerotic and degenerative types, have been reported by Van Slyke and associates (70) and the effect of renal disease upon urea clearance generally discussed by Peters and Van Slyke (71).

Hayman and Johnston (72) have compared the urea clearance with estimates of the number of glomeruli in one kidney after postmortem injection. They considered that in cases of arteriosclerotic Bright's

disease and probably in chronic hemorrhagic Bright's disease, reduction in urea clearance was associated with a decrease in the number of injected glomeruli. In pneumonia and pyelonephritis, urea clearance was greatly reduced in the presence of a normal number of glomeruli. Hayman, Martin and Miller (73) have recently reported similar studies on this same problem. Seventy-nine patients were studied. Nineteen showed no evidence of renal disease during life and none or slight was found at autopsy. These served as controls. There were 2 patients with sub-acute and 7 with chronic glomerulonephritis. Thirty-nine patients had arteriolar nephrosclerosis of varying degree. Twelve patients had low renal function during life but on postmortem examination only slight changes were found and the number of glomeruli was normal. They found poor correlation between urea clearance and renal weight. However, the mean urea clearance fell with a decreasing number of glomeruli but not directly, since the clearance was reduced more rapidly then the number of nephrons. In contrast to this is their observation, upon dogs, whose renal mass had been reduced by sub-total nephrectomy, that the percentage reduction in clearance was less than the reduction in the number of glomeruli. In certain cases of acute infections and jaundice, the clearance they found might be markedly reduced in spite of a normal number of glomeruli showing no significant histologic changes.

Cullen, Nelson and Holmes (24) have reported urea clearances of 15 children during the acute stage of and convalescence from hematuric nephritis. The values were reduced but in a majority of the cases the clearance had returned to normal within a month after cessation of symptoms. In a group of 78 children with a history of acute hematuric nephritis, the distribution and means of the urea clearance values coincided with those cases having no history of renal disease. Apparently acute nephritis leaves little or no residual renal damage in children.

Chasis and Smith (74) have determined inulin and urea clearances in 7 patients during a first attack of diffuse glomerulo-nephritis and in 15 patients with chronic nephritis. In 3 cases during the acute diffuse nephritis, the inulin clearance was markedly reduced. In 13 of the cases of chronic nephritis the inulin clearance was greatly reduced. The changes in urea clearance paralleled those in glomerular filtration and there was no evidence of increased tubular reabsorption of urea. As a matter of fact, tubular absorption was impaired because the U/P ratio in the cases of nephritis fell as low as 2, whereas in the normal it has never been observed by these workers to fall below 6 and frequently

could not be reduced below 10. This indicated impairment in the capacity to absorb water and as the U/P ratio fell from 10 to 2, the reabsorption of urea decreased and the urea/inulin clearance ratio rose toward 1.0. Arkin and Popper (75) have reported that there is less tubular absorption of urea in cases of acute glomerulonephritis, benign hypertension and nephrosis. However, they used endogenous creatinine clearance to measure glomerular filtration and this has been found to exceed the inulin clearance in kidney disease. Miller, Alving and Rubin (55) have determined inulin clearances in patients with nephrosis, chronic and subacute glomerulo-nephritis and essential hypertension. They were made at plasma levels of inulin from less than 5 to 96 mgm. per cent. Since the clearances did not seem to be affected by the level of plasma inulin, there probably was no tubular excretion or absorption of inulin. In the cases of chronic glomerulo-nephritis the inulin clearance was moderately to greatly reduced. There was less reduction in the cases of nephrosis and perhaps none in those of hypertension. Chesley and Chesley (76) have studied renal blood flow, by means of the diodrast clearance, in 37 women with hypertension and renal impairment occurring separately and together. Hypertension sometimes occurred with the total renal blood flow within normal limits. In other cases, the deficit in renal blood flow was only slight. However, in the majority of patients with essential hypertension the diodrast clearance was considerably reduced below the average normal. Such deficit in total renal blood flow seemed often to be associated with efferent glomerular arteriolar constriction and consequent, high filtration fraction which might maintain, in their opinion, normal renal function as determined by other tests. In the hypertension seen in women who had toxemia of pregnancy as the initial phase of the hypertension, the filtration fraction was usually normal. They suggested that the glomerular capillaries rather than the efferent arterioles were the site of resistance to blood flow in these cases. The urea clearance often paralleled the renal blood flow. The exception appeared in those cases where efferent glomerular arteriolar constriction existed. In 70 subjects the coefficient of correlation between the urea clearance and blood flow was 0.79. These workers decided that in most cases the renal blood flow could be predicted within 15 per cent from the urea clearance.

In a series of hypertensive subjects Smith, Goldring, Chasis and Ranges (77) found invariably that the renal blood flow was less than normal values and the filtration fraction greater than was observed either in the normal subject or in subjects with chronic glomerulo-

nephritis of the same degree of renal impairment. Hypertensive subjects in whom the renal mass was not perceptibly reduced, although showing absolute renal ischemia, had a filtration rate and urea clearance which were normal, probably in part because of the increased tone of the efferent glomerular arteries and in part because of increased systemic arterial pressure. In hypertensive subjects in whom the excretory renal mass was markedly reduced the renal blood flow and filtration rate, although reduced absolutely, might be relatively increased when referred to the mass of the residual normal tissue.

Corcoran and Page (78) found that the slow intravenous infusion of preparations containing renin or angiotonin into conscious, trained dogs with only one kidney and it explanted subcutaneously or with both kidneys located subcutaneously resulted in increased arterial pressure, reduced renal blood flow and an increase in the extraction or filtration fraction of inulin from the blood. These results indicated that the efferent arterioles of the nephrons had increased tonus. These substances apparently produce in the dog's kidney a condition quite similar to that observed in clinical hypertension.

CONCENTRATION AND DILUTION TESTS. *Relationship of urine volume to glomerular filtration.* The specific gravity of urine is an expression of the concentration of its dissolved materials. This concentration is determined by the volume of water permitted to reach the urinary bladder, the total amount of urinary solids produced by bodily metabolism and the relative amounts of these solids which escape absorption by the renal tubules. The urine volume is determined normally by the relative amount of water reabsorbed by the tubules and not by changes in the filtration rate (2). Under physiological conditions the filtration rate and urine flow can be varied over a wide range of variation with no evidence that the two factors are dependent upon each other (13, 19, 79). The important rôle of tubular absorption of water in regulating urine volume is seen in a comparison of the glomerular filtration rate in man of about 120 cc. per minute to the urine volume of about 1500 cc. per 24 hours. Furthermore the xanthine and mercurial diuretics apparently increase urine flow independently of any effect upon the filtration rate (2).

Tubular reabsorption of water. Water reabsorption may occur throughout the length of the tubule. Richards found that in the frog the chief site of water absorption was in the proximal part of the distal tubule. He also obtained evidence indicating that water absorption occurred to some extent in the proximal tubule. In some experiments with phlori-

zinized frogs, it appeared that 20 per cent of the water filtered underwent absorption in the proximal tubule (80). In a survey of the comparative physiology of the kidney, Marshall has brought forth the evidence that the thin segment of the loop of Henle plays an important rôle in water absorption. It is only in those animals possessing this structure or some semblance of it that a hypertonic urine is produced. This is restricted to mammals and some of the birds (81). Furthermore Burgess, Harvey and Marshall (82) found that the anti-diuretic factor exerted its effect only in animals possessing the thin segment of the loop of Henle.

The polyuria of diabetes insipidus indicates a failure of part of the physiological mechanism influencing the tubular absorption of water. Fisher, Ingram and Ransom (83) in a very comprehensive review of this problem express their concept of its pathological physiology. "The supraoptico-hypophyseal system regulates the secretion of the antidiuretic hormone by the neural division of the hypophysis . . . the neural division includes the infundibular stem and median eminence. The interruption of the supraoptico-hypophyseal tracts in the hypothalamus causes the neural division to become atrophic and functionally inactive and leads to a deficiency of the antidiuretic hormone in the organism. Likewise, section of the stem high enough to cut all of it and the median eminence away from the hypothalamus brings about a similar atrophy and deficiency. Extirpation of the neural division in all its parts leads to the same hormonal deficiency by virtue of the fact that it removes the site of formation of the antidiuretic principle." Inasmuch as hypophysectomy does not produce polyuria, the above workers consider that the anterior lobe controls the diuretic processes in the body not through a specific diuretic hormone but rather through its general control over metabolism and activity. These workers speak of diabetes insipidus as being caused by a disturbance of the equilibrium normally obtaining between the neural division and the pars anterior.

Verney's experimental studies have indicated to him that the hypophysis is the chief regulating influence in the maintenance of water balance. The inhibitory effect of emotion, muscular activity, pain and fear upon urine flow he considered as due to the pituitary mechanism. The renal nerves and adrenalin were shown not to be a factor in his experiments(2).

On the other hand, the results obtained by Fee and Newton and Smirk (2) indicated that the pituitary was not essential for the control of water diuresis. In decerebrate, hypophysectomized dogs and cats the course of water diuresis was very similar to the control animals

possessing the pituitary mechanism. Furthermore, they showed that when diuresis was obtained more than once after hypophysectomy, the second or third diuresis showed no decrease either in the delay of onset of diuresis or in the delay in attaining the maximum urine flow. They concluded that the delay in onset of diuresis after water administration is independent of the anti-diuretic factor of the pituitary, which conclusion is opposed to Verney's concept that the delay in onset of diuresis was due to the removal or nullification of the anti-diuretic hormone.

Furthermore, the concept that the variable concentration of the anti-diuretic hormone in the blood regulates the degree of water absorption by the tubules under normal physiological conditions fails to explain one important fact. Under the conditions of either extreme water diuresis or diabetes insipidus the urine volume hardly exceeds 20 per cent of the volume of glomerular filtration. Smith (2) has designated the 80 per cent which is always normally absorbed as the obligatory reabsorption. This may be due to the increased colloidal osmotic pressure of the plasma which results from the glomerular filtration and the lowered hydrostatic pressure in the peritubular capillaries. The remaining 20 per cent, the facultative reabsorption, Smith considers to be under the influence of the anti-diuretic hormone. It is the removal of this fraction which results in a hypertonic urine.

Effect of the urinary constituents upon specific gravity. The relative influence of the different urinary constituents on the specific gravities obtained in the concentration tests has been considered by Alving and Van Slyke (84). On the basis of data given in Landolt-Börnstein's "Tabellen" of the effect of those various inorganic salts, which are found in the greatest amounts in urine, upon the specific gravity of water, they calculated that in a urine of specific gravity 1.030 about $\frac{3}{4}$ of the rise above that of water might be attributed to the mineral salts and about $\frac{1}{4}$ to the urea. The ions SO_4, HPO_4 and H_2PO_4 would be expected to have a much greater effect in elevating specific gravity than similar concentrations of other substances found in urine. An experimental study of this problem has been reported recently by Price, Miller and Hayman (85). These workers established the generalization that the specific gravity of a urine could be calculated if the chemical analysis of its major constituents were known. They prepared artificial urines, approximating natural urine in composition and, using data from Landolt-Börnstein or International Critical tables, calculated the specific gravity of such urines. The maximum difference between calculated and observed specific gravity in 5 cases was 0.0007 and averaged 0.0004.

Also after a weighed amount of solid was dissolved in urine, the average difference between observed and calculated specific gravity was 0.005. Furthermore, when a concentrated urine was diluted with water, specific gravity increment was always a linear function of the degree of dilution.

The above workers determined the composition of the urine of human subjects receiving diets of 40, 50, 100 and 110 grams of protein. In urine from subjects on a low protein diet, urea accounted for 15 to 20 per cent, chlorides for 25 to 30 per cent, sulfate and phosphate together 15 to 25 per cent, bicarbonate 1 to 5 per cent and creatinine 1 to 2 per cent of the observed specific gravity. On a high protein diet, urea, sulfate and phosphate contributed a slightly higher proportion to the specific gravity. The undetermined fraction of specific gravity comprised 10 to 30 per cent and in the same urine the excretion of undetermined solids amounted to 10 to 15 grams daily or from 10 to 30 per cent of the total solids. The undetermined solids are composed of organic substances, are low in nitrogen, are dialyzable and are largely of endogenous origin. The various coefficients proposed for estimation of total solids in urine from specific gravity were found by Price, Miller and Hayman to be valid only for urines of the same relative composition.

The studies of Gamble, McKhann, Butler and Tuthill (86) upon the relation of urine volume to the amount of various urinary substances excreted by rats given free choice of water is pertinent. The water requirement established for the individual substances such as Na, K, Cl, HCO_3, H_2PO_4 and SO_4 remained additive when mixtures of them entered the urine. In the presence of urea, water expenditure was found to be much less than the sum of the requirements for urea and the accompanying substances as separately determined. This economy was not demonstrated with urinary glucose or galactose. The kidney may not be the only factor in these experiments making for water economy in the excretion of urea inasmuch as water consumption was not controlled. However, this behavior of urea would tend to elevate the specific gravity of the urine.

Factors affecting the excretion of electrolytes. The renal excretion of water and inorganic salts has been reviewed by Peters (87) especially in relation to the homeostasis of body fluids. The urinary excretion of sodium, potassium, calcium, chloride, phosphate and sulfate was discussed by Smith (2) in 1937, and later brought up to 1939. Most of the electrolytes in the plasma act like "threshold" substances, in that they are largely retained when their plasma concentration is low and are rapidly excreted when the plasma concentration is elevated. The uri-

nary excretion of chloride is influenced by the level of plasma chloride. In man the excretory rate is about 5 mgm. of NaCl per hour when the plasma Cl is below 95 mM per liter. Above this level the Cl excretion increases rapidly. Water diuresis increases the loss of both Na and K. Diuresis caused by NaCl increases the loss of K and KCl similarly affects the excretion of Na. For potassium, apparently there is a renal threshold of about 3.5 mM per liter and above this plasma concentration, K is rapidly excreted (88). Hall and Langley (89) found that the potassium clearance in 3 normal subjects was constant over a wide range of urine flow but below a flow of 0.6 cc/min./$\overline{M}^2$ the clearance fell sharply.

Adrenal insufficiency affects the urinary excretion of Na and K. Loeb and associates (90) conducted balance studies before and after adrenalectomy in 3 dogs. An increase in blood potassium was not obviously correlated with changes in the K balance. The balance of Na showed a striking loss of Na during development of adrenal insufficiency. After adrenalectomy, the urine volume, and both the concentration and total amount of urinary Na were increased. The behavior of Cl paralleled that of Na but its loss was not equivalent to that of Na. Cessation of the injections of cortical extracts in adrenalectomized dogs is associated with increased excretion of Na which reaches its maximum in 48 to 72 hours. The concentration of Na may be nearly doubled in this period even though the urine volume is greatly increased. After 3 days, the urinary excretion decreases because, according to Harrop (91), of depletion of the body's reserves of Na. Anderson, Joseph and Herring (92) reported that 24 hours after adrenalectomy in rats there is an increased urinary excretion of radio-active Na and retention of radio-active K. Also, adrenalectomized rats receiving 10 per cent NaCl, at first excrete administered radio-active Na like normal control rats, but later show Na retention. The excretion of radio-active K was the same as the controls (93). Harrison and Darrow (94) determined the volume of glomerular filtrate and simultaneously the urine/plasma ratios of Na, K and P in the adrenalectomized dog under various conditions. They concluded that following adrenalectomy the renal tubules fail to reabsorb Na adequately from the glomerular filtrate at a time when the concentration of Na in the plasma is low and fail to excrete K and phosphate when these ions are abnormally concentrated in blood plasma. The excretion of these ions was expressed in terms of the ratio of their concentration in urine (U) to that in plasma (P). However, the U/P ratio does not indicate the magnitude of the tubular reabsorption of these ions because it does not consider the rate of glomerular filtration

and the per cent of the filtered water which is reabsorbed. Using their data as given, this writer has calculated the clearances of creatinine, Na and K and the per cent of the filtered ions that is absorbed by the tubules. These calculations showed that in adrenal insufficiency the percentage of the filtered K which was reabsorbed was either about the same as normal or much less than normal. Administration of Na or Na plus the cortical extract greatly reduced the percentage reabsorption. This effect together with the increased glomerular filtration would explain how therapy corrects the plasma accumulation of K. Adrenal insufficiency did not materially change the tubular reabsorption of Na, inasmuch as with one exception 99 per cent or more of the filtered Na was reabsorbed. This result is not in agreement with the finding that the urinary Na is increased in adrenal insufficiency. The reason for the discrepancy is not apparent. The administration of Na in adrenal insufficiency reduced the percentage reabsorption of Na. In this respect the tubules of the adrenalectomized dog respond as in the normal dog.

Mainzer (95) found that in patients remaining in bed with a normal intake of food, the ratio of Na:Cl varies greatly in the urines collected in a 12 hour day as well as 24 hour periods. Tests carried out over a long period revealed that the excretion of Na and Cl occurred in equivalent quantities. Clinical use of this ratio according to Mainzer must be limited to prolonged observation periods.

Since Smith's (2) discussion of the excretion of sulfate two papers have appeared on the subject. Goudsmit, Power and Bollman (96) found in the dog that the clearance of the naturally occurring concentrations of sulfate in the plasma approximated 8 per cent of the creatinine clearance, indicating that about 92 per cent of the filtered sulfate is absorbed by the tubules. Increasing the rate of urine flow decreased the fraction which was reabsorbed. After the intravenous injection of sulfate its clearance rose markedly and at concentrations of 180 to 200 mgm. per cent it was about 90 per cent of that of creatinine. All or nearly all of the sulfate in the serum of dogs could be considered filterable through collodion membranes. Bjering and Øllgaard (97) found the endogenous sulfate clearance in normal persons to average 37. They reported that it increased with the administration of sulfate, approaching a constant value between urea and creatinine clearances. It was generally lower in patients with kidney disease, corresponding with the fall in creatinine and urea clearances. They also reported that the straight line curve obtained by plotting the concentration of blood sulfate against the sulfate clearance could not be extended through zero

concentration of blood sulfate but crossed the concentration at 0.8 to 0.9 mgm. per cent. This type of curve they interpreted as indicating that this much of the blood sulfate was not filterable at the glomerulus. They state that after correcting for this non-filterable fraction the sulfate clearance has the same numerical value as those reported for inulin clearance. Such a statement without the simultaneous determination of inulin and sulfate clearances is hardly acceptable. Furthermore, Hayman (98) found no evidence from similar curves that part of the blood sulfate in the human was not filterable at the glomerulus.

The specific gravity of urine is therefore the resultant of a number of physiological processes carried on within the kidney. To these renal factors should be added the extra-renal factors pointed out by Barker (99) such as impairment of the systemic circulation and the hydropigenous states of salt retention, dehydration and inanition. Lewis and Alving (25) found that the maximum specific gravity declined with those ages above 40 years. At 40, the specific gravity was 1.030 and at 90 years it was 1.023. Manchester (100) has reported a diurnal rhythm in water and mineral excretion. The urine volume and the urinary Na, K and Cl were greater during the day than at night. Only slight fluctuations were noted in urinary phosphate, sulfate, titrable acidity and ammonia. The water balance was found to be negative during the day and positive at night. The rise in specific gravity of night urine he decided was largely due to increased concentration of the nitrogen compounds. In dehydration the diurnal rhythm in Na excretion was temporarily reversed.

Specific gravity of urine in nephritis. In nephritis the specific gravity of the urine undergoes changes which have been considered of great importance in evaluating renal function in this condition. The specific gravity of urine in acute nephritis varies, apparently depending upon urine volume. With oliguria the specific gravity is usually 1.020 higher. The histological picture usually shows much ischemia of the glomeruli and swollen endothelia of the glomerular vessels (101). This of course greatly lowers filtration pressure in the glomeruli so that the rate of glomerular filtration is much reduced. Reference has already been made to the experimental finding of reduced glomerular filtration in acute nephritis. Presumably the slower rate of filtration would allow more time for the tubular reabsorption of water leaving a more concentrated urine. In chronic nephritis there is polyuria and the specific gravity of the urine is not only reduced but does not show the variations found under normal conditions. The possible mechanism of these uri-

nary changes interested Bradford (102) because although many nephrons are rendered functionless by disease, those remaining should excrete a normal urine. He found that surgical excision of portions of both kidneys in dogs resulted in a great increase in urine volume. If one-third of the total kidney weight is left, the animal remains in good health, the urine volume is increased and the animal cannot concentrate the urine. Using a heart-lung and two kidney preparations, Verney (103) reported that ligature of the anterior branch of the renal artery of one kidney resulted in a decreased renal blood flow and no diminution in urine flow. After a short time the urine flow gradually increase above that of the control kidney. In some cases the urine flow was thirty times the control. The chloride concentration of the urine usually did not change but the minute output increased. The concentration and minute output of urea immediately fell but was greater than that expected on the basis of the amount of kidney still being perfused. A similar result was obtained by Verney in the unanesthetized dog by ligature of one renal artery. The urine flow from the normal kidney within an hour increased over its control rate and within 3 hours was nearly three times as great as that of both kidneys during the control observation. Injection of pituitary extract did not diminish the polyuria.

Rytand (104) statistically studied the data obtained in a number of nephritic patients. He reported a curvilinear relationship between specific gravity of the urine and the Addis ratio. The kidney was able to excrete a concentrated urine until the ratio had decreased to 80 per cent of the normal. Below this value the urine's specific gravity rapidly decreased until the ratio was 30 per cent of normal.

Hayman, Shumway, Dumke and Miller (105) have recently reviewed the literature of, and reported their own experimental study of hyposthenuria, or the inability to concentrate the urine. They found that hyposthenuria in dogs could be produced in a number of ways. These included reduction in kidney mass, uranium poisoning, ureteral obstruction, renal denervation and low protein diets. The reduction in functioning kidney mass was accomplished by surgical removal of about one-third of one kidney and later complete removal of the remaining kidney. The concentration tests, after complete recovery, showed a urine of increased volume and decreased specific gravity. It was found that these dogs could excrete a concentrated urine, under certain conditions such as increased concentration of plasma colloids, low blood pressure and intravenous injections of sodium sulfate. Adrenal cortical

hormone and pituitrin were without effect in causing such kidneys to concentrate the urine. These workers thought that the polyuria and hyposthenuria in these dogs were due to increased blood flow to the remaining nephrons with a resultant increased volume of glomerular filtrate per nephron. The return to a normal concentrating capacity effected by a reduction in blood pressure and the curvilinear relationship found between creatinine clearance and the per cent of glomeruli remaining post-operatively in these dogs are in agreement with this idea but unfortunately these workers did not determine the renal blood flow or calculate the per cent of the filtered water which underwent tubular absorption in the normal and post-operative conditions.

In uranium poisoning, presumably with a predominantly tubular lesion, the inulin, creatinine and urea clearances were reduced and there was an increased volume of dilute urine. The lowered clearances were attributed by these workers to failure of the damaged tubules to prevent diffusion of these substances into the peritubular capillaries. A urine of high specific gravity was not obtained from dogs having renal tubular damage, probably because of the back diffusion.

Injection of pituitrin enabled the denervated kidney to excrete a concentrated urine. The hyposthenuria in dogs with a low protein diet was probably due to the small amount of urinary solids produced by bodily metabolism.

Reference has been made to the study of renal function and the number of glomeruli in the human kidney by Hayman and associates (73). They found upon plotting the maximum specific gravity of the urine against the number of glomeruli which was determined post-mortem in cases of chronic glomerulonephritis and nephrosclerosis, that the specific gravity fell with the number of glomeruli until the latter reached about 750,000 glomeruli per kidney, after which it remained fixed at about 1.010 in spite of further reduction in the number of glomeruli.

A hepatorenal syndrome has been discussed in papers from clinicians. Nonnenbruch (106) has written of a nitrogen hyposthenuria as occurring in cases of liver disease such as catarrhal icterus, vascular congestion, lues, cirrhosis and carcinoma of biliary tract. According to Nonnenbruch, there may be oliguria, hyposthenuria and renal insufficiency or polyuria, hyposthenuria and no insufficiency. Boyce and McFetridge (107) have also written of a hepatorenal syndrome and express the idea that obstruction to the biliary tract damages the liver which then causes oliguria, anuria and death. Both of these clinical papers are characterized by vague, worthless generalities and extreme paucity of scientific

data. A direct, specific effect of the liver upon renal function remains to be demonstrated.

The polyuria, hyposthenuria and relative fixation of the urine's specific gravity, when part of the kidney substance is removed, is a striking physiological reaction. Verney (103) refers to the normal condition as one in which a part of the organ inhibits the activity of the other part. He considers that in this response the physiological reserve of the remaining nephrons has been drawn upon. It would appear that the reaction serves a compensatory function but the actual mechanism remains to be elucidated. This reaction of the kidney to a loss of kidney substance may explain the polyuria and the reduction of the specific gravity of urine in chronic nephritis.

As the nephritic process advances on the kidney, the maximal concentrating capacity of the kidney is reduced. On the basis of the preceding discussion, a number of factors might be involved. Since the range of specific gravity of the urine is between isotonicity and hypertonicity, the water absorbing capacity of the thin segment of the loop of Henle might be greatly impaired. In support of this idea is the reported failure of pituitrin to prevent the polyuria of chronic nephritis. There might be a failure on the part of the tubule to prevent the diffusion of the urinary solids into the peritubular capillaries. Hayman and coworkers (105) considered that this occurred in the experimental nephritis produced by uranium. It has been reported that sulphate retention in the blood occurred before that of urea. This might be due to increased absorption of the sulfate but it awaits experimental study. Impairment in the capacity of the kidney to dilute the urine probably indicates reduction in the ability of the tubules to reabsorb the electrolytes. Peters, Wakeman and Lee (108) found that in advanced nephritis both base and chloride were excreted in the urine when serum Cl had fallen below the level which in a normal individual determines achloruria. Arkin and Popper (75), using the endogenous creatinine clearance as a measure of glomerular filtration, reported that there is less tubular absorption of urea in patients with damaged kidneys. When the maximum specific gravity reaches 1.010 the renal tubules have practically ceased to exercise any selective rôle in the reabsorption. Since this value approximates that of an ultra-filtrate of blood plasma, namely 1.007, if the tubules are reabsorbing any of the glomerular filtrate, it is solely by physical diffusion, and the composition of the absorbate would be about the same as the glomerular filtrate. Failure of the nephritic kidney to lower the specific gravity below 1.010 in the dilution test indicates

that the tubules cannot selectively reabsorb salts. In other words when the specific gravity of the urine becomes relatively fixed at 1.010 the tubules have become merely inert tubes through which a fluid resembling an ultra-filtrate of blood plasma may pass either to the collecting ducts or to the peritubular capillaries.

Comparison of urea clearance with some of the other tests of renal function. Van Slyke and associates (109) have reported that in patients with diminishing renal function, the urea clearance shows evidence of the diminution sooner than does the blood content of creatinine or urea or the phenolsulfonephthalein. Alving and Van Slyke (84) have compared the results of concentration and dilution tests with the urea clearance in nephritic patients. They concluded that the concentration tests, done with proper care, were sensitive for qualitative detection of damaged renal function. For measuring the extent of renal damage, however, the concentration tests did not appear to be suitable. Although the extent of fall in urine concentration showed statistical correlation to the urea clearance, the disagreement in given cases might be extreme. A patient may have practically recovered from nephritis, with a normal urea clearance and yet excrete urine of specific gravity 1.009 to 1.012 which is the same as that of a patient in terminal uremia with 3 to 5 per cent of normal clearance. Freyberg (110) has discussed the choice of tests of renal function. He compared in patients with hemorrhagic nephritis, urea clearance, the P.S.P. for 15 minutes and the concentration tests. He decided that generally a parallelism existed between the three tests. In the initial stage of nephritis concentrating power was lost and during recovery the urea clearance became normal while concentration was still impaired. In arteriosclerotic Bright's disease there was more difference between clearance and concentration. In 4 cases, clearance was lowered with normal concentration. In 38 cases there was normal clearance and impaired concentration. In 4 cases the clearance was elevated and concentration impaired. He concluded that the concentration test was more sensitive in this series of cases in whom the renal damage was less severe. In 12 of these cases there was a low dye excretion with normal concentration. Perhaps one factor which operates to make urea clearances appear less sensitive than the concentration test is the great variation of urea clearance in normal subjects. Although 75 to 125 per cent is generally considered normal, a subject may have lost 50 per cent of his clearance and yet be considered in the normal range. This individual variation does not appear in carefully done concentration tests. Clearances seem to have the greater

value when it is desired to measure renal function at intervals over a considerable period of time.

This survey of the factors influencing renal function did not yield much information about the physiological regulation of renal activity. Although many factors may influence the various aspects of renal activity the fundamental control is yet to be learned. Even the hypothalmico-hypophyseal mechanism apparently is chiefly concerned with raising urine from isotonicity to hypertonicity or with the tubular absorption of about 20 per cent of the filtered water. The excretion of the electrolytes and of glucose has a certain kind of regulation by the threshold mechanism, that is, when the amount filtered per minute exceeds the amount reabsorbed by the tubules, the excess is excreted. It might be that renal function proceeds at the maximum capacity. However, Richards and Schmidt (111) directly observed intermittence in glomerular activity of the frog. Khanolkar (112) injected carmine or hemoglobin intravenously into rabbits and upon microscopic examination found more of the injected substance in some glomeruli than others. Hayman and Starr (113) intravitally stained the glomeruli of rabbits by injecting Janus green B into the aorta. Wide variations in the number of glomeruli open to the dye occurred spontaneously. These workers reported that caffein and salt solution increased the number of open glomeruli, whereas adrenalin and CO_2 inhalations usually reduced the number of open glomeruli. On the other hand, White (114) has reported observations on glomerular perfusion made on 17 kidneys of 11 dogs and 13 kidneys of 7 rabbits following injection of a carbon suspension into the renal artery. He found that either all the glomeruli were injected or that the distribution of the uninjected glomeruli was such as to favor the idea that failure of injection was due to the distribution of ink in the larger pre-glomerular arteries rather than to closure of glomeruli. The question of intermittent, sub-maximal or maximal activity of the nephrons remains confused. From the physiological viewpoint this is an important problem because if renal activity proceeds at a maximal rate, a search for factors regulating this activity would likely prove fruitless. In applying the tests of renal function to the diseased kidney, one might consider that part of any impairment of function might be due to renal structural changes. Hayman (115) upon perfusing kidneys removed at autopsy, found that those kidneys of benign arteriolar sclerosis, acute and chronic diffuse glomerulonephritis, yielded less perfusate at various perfusion pressures than did normal kidneys. The kidneys showing only degenerative changes gave the

same volume of perfusate as the normal. In addition to these structural changes, there may be purely functional changes which might be responsible for the renal impairment. Some of the more recent clearance methods may make possible a differentiation between these two types of causes for renal impairment.

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ANAPHYLAXIS

CARL A. DRAGSTEDT

Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago

An anaphylactic reaction may be defined as a reaction that is produced in an animal by the administration of a foreign substance, the reaction occurring in an animal sensitized to that substance by a preceding suitable exposure, the nature and severity of the reaction depending upon the kind and degree of the sensitization of the animal and not upon any toxic properties of the foreign substance, so that the reaction may be said to be characteristic for the animal rather than characteristic for the foreign substance. The anaphylactic reaction is one of the expressions of an antigen-antibody reaction. Correspondingly there are numerous problems concerning the nature of the antigens and antibodies involved, methods of sensitization and of inducing the reaction, the phenomena of desensitization and refractory state, passive transfer of the sensitized state, etc. These may be considered as immunological problems and they will not be dealt with in this discussion except as they impinge upon the argument. The explosive and violent character of the anaphylactic reaction, with marked effects manifested in the respiratory, circulatory, glandular and smooth muscle systems, presents the problem of the proximal pathogenesis of these symptoms that is no less intriguing than the more fundamental immunological problem. Even with this narrowing of scope the literature is too extensive to permit more than an appraisal of the present status and the work that has seemed to be pertinent thereto.

Concurrently with the accumulation of a wide variety of experimental observations regarding the conditions under which an anaphylactic reaction might be obtained, numerous theories to explain the manifestations were elaborated (14, 33, 39, 55, 56, 58, 67, 71, 73, 79, 88, 89, 90, 91, 102, 130, 138, 149). Space does not permit a detailed consideration of either these theories or the varieties of evidence submitted in their support. Suffice it to say that a mass of evidence has accumulated to establish that the anaphylactic reaction is indeed the product or biproduct of an antigen-antibody reaction. Furthermore there is sub-

stantial evidence that it is the antibodies attached to fixed tissue cells which are most concerned in this reaction. This evidence is most abundant and convincing in the cases of the dog and the guinea pig. As will be referred to later, it may not be so true for the rabbit and perhaps other animals. Manwaring (82), Pearce and Eisenbrey (98), Coca (21), and Fenyvessy and Freund (52) have shown that the blood of a sensitized animal can be removed and replaced with the blood of a normal animal without impairing the sensitivity of the animal. Various workers (see 75 for references) have reported that there is a necessary latent period for the passive sensitization of normal animals by the injection of antiserum, which fact is difficult to explain if antigen and antibody react in the circulating blood, but fits in well with the assumption that a latent period is necessary for the antibody to enter or become attached to the fixed tissue cells and thereby render them sensitive. Schultz (115) and Dale (27) demonstrated that isolated tissues (intestine, uterus) of sensitized animals, washed as free as possible from blood, show a specific or anaphylactic contraction when antigen is added to the bath in which they are suspended. Finally, evidence has been presented by Weil (139) and by Dale and Kellaway (29, 30) indicating that an abundance of free antibody instead of favoring the occurrence of a reaction actually interferes with the anaphylactic reaction presumably by binding the antigen before it can reach the sessile receptors within the fixed tissue cells.

Despite the apparently incontrovertible mass of evidence for what is usually termed the cellular hypothesis there have been presented both rationalizations and experimental evidence to support a humoral concept, namely, the intravascular formation of toxins which in turn produce the symptoms. Wells (143) has stated that there are no pathological changes in anaphylactic shock that are inconsistent with the possibility that some poison similar to the alkaloidal poisons could be concerned. Vaughan and Wheeler (129, 130) demonstrated that from almost any protein a highly poisonous substance can be produced by cleavage under suitable conditions and that this toxic fraction produces effects in animals closely resembling anaphylactic shock. Biedl and Kraus (17) pointed out that the intravenous injection of peptone solutions produced reactions virtually indistinguishable from anaphylactic shock, and Dale and Laidlaw (31) reported that the pharmacological effects of histamine, a derivative of histidin, are strikingly similar to those seen in anaphylaxis. Correspondingly a number of workers had reported apparent exceptions to the statement that a latent period is

essential for passive sensitization and to the statement that simultaneous injection of antigen and antibody into a normal animal does not provoke anaphylactic symptoms. Reference to these exceptions will be made later in the section devoted to anaphylaxis in the rabbit.

There was thus an attractive if not very solid basis for the theory that when a protein antigen is injected the second time it is split into toxic degradation products such as Vaughan's poison, peptone or histamine by the proteolytic ferments which formed as the result of the first injection. As early as 1908, however, Wells (142) had pointed out the extreme improbability of the view that the minute amount of egg albumin that suffices to produce almost immediate symptoms in a sensitized guinea pig could in the short time available undergo proteolysis with the liberation of an adequate amount of toxic degradation products. The validity of this criticism was such that it was necessary either to discard the toxin theory or look elsewhere for its origin. Jobling and Petersen (67) evolved the theory that the union of antigen and antibody in the blood somehow removed from the sphere of action the natural antitryptic constituent of the plasma so that the protease, which it normally holds in check, becomes effective, digesting the proteins of the plasma itself with the formation of toxic cleavage products. While avoiding the quantitative embarrassment involved in considering the antigen as the matrix from which the toxin is derived, this theory had an equally difficult time factor with which to contend and no substantial evidence for it was forthcoming.

Many workers have assumed that the cellular theory and the various humoral theories were mutally exclusive so that as the evidence accumulated that the reaction took place in or on the fixed tissue cells, this evidence was construed as contradicting the humoral or anaphylatoxin hypotheses. The pure cellular theory, however, has no satisfactory explanation for the mechanism by which the antigen-antibody reaction produces the phenomena of anaphylactic shock. When Simonds (120) pointed out that the dominant symptoms of bronchospasm in the guinea pig, circulatory failure in the dog, and cardiocirculatory failure in the rabbit, could all be harmonized on the basis of a spastic contraction of strategically located smooth muscle (in the bronchioles of the guinea pig, in the hepatic veins of the dog, and in the pulmonary arterioles of the rabbit) a distinct step was made in demonstrating that the fundamental reactions in all animals were probably identical. There still remained, however, the question of how the antigen-antibody reaction made the smooth muscle contract, and in addi-

tion how were the phenomena of capillary dilatation and permeability, increased secretion of various glands, changes in the coagulability of the blood, etc., to be harmonized and explained? The crux of the problem from a mechanistic point of view would seem to be the determination of whether the antigen-antibody reaction per se (by producing disturbances of colloidal equilibrium, intracellular precipitation analogous to that seen in vitro, etc.) suffices to explain the varied symptoms, or whether there are in fact any anaphylactic toxins specifically related to the reaction and adequate to account for the symptoms.

Physiological studies of the reactions in the dog, the guinea pig and the rabbit have yielded answers to some of these questions and these observations will be discussed separately in that order although of course there has been no corresponding sequence in their experimental development.

Anaphylaxis in the dog. The analysis of the anaphylactic reaction in dogs began with the work of Richet (106). He used water extracts of the tentacles of sea anemones, actinia and mussels, and also a vegetable toxalbumin, crepitin. Although all these extracts were poisonous, and in adequate dosage caused death, Richet noted that sublethal doses, which produced only mild symptoms in normal dogs, would produce violent symptoms and death when injected intravenously into dogs which had received a similar injection two or three weeks previously. A possible cumulative action of the poison was ruled out by the observation that reinjections at intervals of three to five days produced only a moderate effect. The symptoms observed were dyspnea, vomiting, salivation, general weakness, diarrhea and a marked fall in blood pressure. Biedl and Kraus (17) as well as Arthus (6) extended these observations. Biedl and Kraus reported that the fall in blood pressure accompanied and paralleled in degree the severity of the other symptoms. They noted also that while anesthesia prevented the vomiting, diarrhea, etc., it did not prevent the fall in blood pressure, from which they concluded that these reactions were probably a sequel to and less important than, the vascular reaction. They reported that the fall in blood pressure was not due to a weakened heart, but was due to a peripheral vasomotor paralysis. Their evidence for this conclusion was that they found epinephrine to be ineffective in restoring the pressure while barium chloride was much more effective. Eisenbrey and Pearce (51), by direct registration of cardiac activity, and Robinson and Auer (108) by electrocardiographic studies confirmed the conclusion of Biedl and Kraus that the fall in blood pressure was due to vascular and

not cardiac influences. Pearce, Karsner and Eisenbrey (99) demonstrated that with the decrease in arterial pressure there was a decrease in the volume of the kidney, intestine and spleen, while there was a large accumulation of blood in the liver.

Manwaring (82) reported that ligation of the aorta and vena cava above the diaphragm prevented the development of anaphylaxis in dogs and that therefore some infra-diaphragmatic organ was essential to its occurrence. As removal of the intestines, stomach, kidneys, adrenals and spleen had no effect, while short circuiting the liver out of the circulation did prevent the reaction, he concluded that the acute anaphylactic reaction in dogs was an explosive autointoxication of hepatic origin. He performed some cross circulation experiments between anaphylactic and normal animals and as a fall in blood pressure occurred in the normal animal when the anaphylactic reaction was induced in the sensitized animal, he concluded that some blood borne toxic agent, apparently of hepatic origin, was concerned in the reaction.

That the integrity of the liver is indispensable for the development of the complete symptomatology in the dog was further substantiated by the observations of Voegtlin and Bernheim (131) who failed to get anaphylactic shock in dogs after the production of an Eck fistula and ligation of the hepatic artery, by Denecke (35) with similar experiments, and by Simonds and Brandes (121) who found that anaphylactic shock did not occur in dogs when the antigen was injected while the circulation through the liver was prevented by occlusion of the hepatic veins, but restoration of the circulation through the liver permitted the reaction to develop. While some anaphylactic manifestations can be induced in the dog which are not apparently dependent upon the liver (34, 136) it has been generally accepted that the major manifestations of shock in the dog are associated with a hepatic reaction.

Weil (140) and Weil and Eggleston (141) failed to confirm Manwaring's experiments which had seemed to indicate the liberation from the liver into the circulation of a vasodepressor substance. In the first place they claimed that cross circulation experiments would of necessity be inconclusive, since it would be obvious that a marked fall in blood pressure in the anaphylactic animal would result in the normal animal becoming partially exsanguinated by bleeding into the anaphylactic animal until their respective pressures were equalized. Secondly, they transfused blood obtained from anaphylactic dogs as well as blood to which antigen had been added and perfused through the liver of a sensitized dog, into normal dogs and reported that such experiments gave

negative results. They concluded that Manwaring's experiments were in error and that no circulating toxin related to the anaphylactic reaction was to be found in anaphylactic dogs. Weil believed that the impounding of blood in the liver mechanically produced by a swelling of the liver cells was adequate to account for the fall of blood pressure in dogs. In 1925 Manwaring, Hosepian, O'Neill and Moy (83) vascularly anastomosed the hind quarters of a normal dog to a sensitized animal and noted that the urinary bladder and intestine of the normal animal contracted when anaphylactic shock was induced in the sensitized animal. Since this could not be explained by the nonspecific effect of the fall in blood pressure of the donor animal it seemed necessary to conclude that some smooth muscle stimulating agent had been elaborated. This experiment did not provide much opportunity for identifying the smooth muscle contracting substance. However, Manwaring, Reeves, Moy, Shumaker and Wright (84) repeated this experiment using the hind quarters of an immunized animal and as they got negative results with this experiment they concluded that the active substance was a sort of secondary antigen, presumably of protein nature, to which the immunized tissues were unresponsive.

That histamine met the requirements for an anaphylactic poison, at least in so far as smooth muscle stimulation, vasodepression, glandular stimulation, dilatation of capillaries, etc., were concerned, had been indicated since the initial studies of the pharmacology of this substance by Dale and Laidlaw. The dramatic suddenness of the onset of the symptoms in anaphylactic shock prompted, however, a hesitancy in believing that adequate histamine could be formed by ferment action from any protein source within the short time elapsing after the injection of antigen and the development of the symptoms. The observations of Lewis (76) on the cause of various capillary reactions occurring in the skin in response to irritations of various sorts led him to the conclusion that they were produced by a histamine-like substance which existed preformed in the living epidermal cells. While histamine had been found in various tissues by a number of workers it was not until 1927 that Best, Dale, Dudley and Thorpe (16) established beyond reasonable doubt that normal living tissues contained histamine, thereby disposing of the argument that it was formed postmortem by bacterial action or autolysis. As a consequence of these findings Dale (28) restated the anaphylatoxin hypothesis, considering the similarities in the symptomatology produced by histamine and by the anaphylactic reaction adequate for the identification of the anaphylatoxin as his-

tamine, and considering the source of the latter as the tissues themselves and not the antigen or plasma proteins, etc.

With attention thus directed to the tissues, Watanabe (135) reported some analyses of the histamine-equivalent of various tissues from normal, sensitized and shocked dogs. He found the liver histamine of 3 sensitized dogs approximately four times as great as that of 4 normal dogs and of 4 recently shocked animals, while he found no changes in the histamine content of other organs. Although Watanabe recognized the temptation to conclude that these findings indicated a discharge of histamine from the liver into the blood during the anaphylactic reaction he was content to call attention to the fact that marked changes occur in the histamine content of those tissues which are concerned in the anaphylactic reaction. He appreciated the marked variations in liver histamine that are to be found in a series of animals and concluded that until it was shown that the marked rise in liver histamine apparently resulting from sensitization was shown to be a regular and specific change and also until it was shown that a decrease was equally regular and specific for the shock reaction, one could not conclude from his findings that there was a liberation of a histamine-like toxin during anaphylactic shock. It is to be noted that he did not follow the changes in liver histamine in any one animal but assumed that group results reflected such changes.

In 1911 Calvary (19) had reported that anaphylaxis in dogs is accompanied by a marked increase in the flow of lymph from the thoracic duct. This was confirmed by Petersen and Levinson (101) and by others. Simonds and Brandes (122) reported that mechanical obstruction of the hepatic veins, which produces a congestion of the liver similar to that seen in anaphylaxis, likewise produces an increase in thoracic duct lymph. Dragstedt and Gebauer-Fuelnegg (42) were prompted by these observations together with the evidence that the anaphylactic reaction in a dog is predominantly a hepatic reaction, to look for an anaphylatoxin in the thoracic duct lymph. They examined the lymph of anaphylactic dogs and found a smooth muscle stimulating agent which was not present normally and which rapidly appeared as shock developed. They (59) investigated the nature of the active substance and reported that it was a dialyzable crystalloid of basic properties, stable to boiling with acid, and inactivated by condensation with diazotized sulfanilic acid. It contracted the guinea-pig intestine, but not that of the mouse, lowered the blood pressure of the atropinized cat and produced histamine-like wheals in human skin.

They believed this evidence adequate to identify it as histamine. In their first experiments Dragstedt and Gebauer-Fuelnegg did not examine the blood regularly because of Weil's previously mentioned negative experiments, and they reported that only occasionally did they find an active substance in the blood similar to that found in the lymph. In connection with control experiments in which various doses of histamine were injected intravenously into normal dogs so that blood and lymph specimens could be studied in parallel fashion with those obtained from anaphylactic animals, it was observed that histamine disappeared very rapidly from the blood (43). Since in the initial anaphylactic experiments in which the blood was examined, samples were taken at what was assumed to be the height of the reaction, it became apparent that many negative results were probably due to the fact that if histamine had been elaborated with the onset of the reaction it had already disappeared by the time the blood sample was obtained. Consequently Dragstedt and Mead (45) reëxamined a large number of anaphylactic dogs, testing the blood for histamine activity at frequent intervals. They then found histamine activity in the blood regularly whenever an appreciable anaphylactic reaction had occurred. Furthermore, they concluded that it was possible by applying the data on the rate of disappearance of injected histamine to the data on the rate of disappearance of histamine activity from the blood of anaphylactic animals, to ascertain how much histamine had been elaborated in the anaphylactic reaction. They concluded from this comparison that the vascular reaction in anaphylactic dogs can be completely accounted for by the amount of histamine thus computed. Dragstedt and Mead (44) also presented further evidence that the active substance was histamine by showing that it is inactivated by histaminase.

Code (24) using a modification of the method of Barsoum and Gaddum (12) for assaying histamine in blood, has confirmed the above evidence for the liberation of histamine during anaphylaxis in dogs. By making quantitative determinations of the concentration of histamine in the blood during anaphylactic reactions as well as after the injection of histamine he has likewise concluded that the amount of histamine liberated during an anaphylactic reaction is adequate to account for the corresponding vascular reaction. It should be added that Code's results also add to the evidence that the active substance is histamine, since Code and Ing (26), using similar methods, have isolated histamine in crystalline form from a large amount of blood.

Dragstedt (40) and Code (24) have both explained Weil's negative

results. Weil obtained blood from anaphylactic dogs at least 20 minutes or more after the injection of antigen, at which times the bloods would have, in most instances, more or less completely lost their activity. Furthermore he transfused the collected blood into normal animals that were anesthetized, but he did not record the blood pressure in these animals. Consequently, even if an occasional specimen of blood that he used had been active, there was no adequate means for Weil to note any effect.

A striking loss of coagulability of dog's blood during anaphylaxis was noted by Biedl and Kraus and by Arthus. This has been the subject of study by a number of investigators (36, 37, 38, 49, 64, 66, 72, 74, 87, 92, 100, 113, 119, 132, 136, 140, 151) and has variously been attributed to an excess of antithrombin, a diminution of thromboplastin, a deficiency of platelets, the transformation of fibrinogen into an incoagulable tautomeric modification, a decreased prothrombin, decreased fibrinogen, etc. The complexities and changing views of the coagulation process in blood are reflected in these various reports. Parenthetically it may be said that certain investigators (73, 87) have thought that anaphylactic shock was due to a transient intravascular coagulation with fibrin clumps acting as emboli and thus producing the reaction, and that the incoagulability of the blood was a sequel to the coagulative phase. Eagle, Johnston and Ravdin (49) demonstrated that the incoagulability of the blood cannot be accounted for by a deficiency of fibrinogen, a platelet deficiency or a prothrombin deficiency, while they found a very marked increase in the anti-thrombic activity of the plasma. Waters, Markowitz and Jacques (136), making use of the observation of Chargaff and Olson (20) that protamine combines quantitatively with heparin, tested blood from anaphylactic animals and reported a marked increase in the heparin titre. Recently Jacques and Waters (66) have reported the isolation of crystalline heparin from the blood of anaphylactic dogs, thus completing the proof that it is the cause of the incoagulability. There is no reason to suppose that it contributes to any of the vascular or other symptoms (Best, Cowan and Maclean, 15). It is interesting to note that Quick (104) on the basis of an indirect method, came to the conclusion that heparin was the cause of the incoagulability of the blood occurring in peptone shock, which, as has been mentioned, is virtually indistinguishable from anaphylactic shock in its symptomatology.

Presumably in dogs, all or the major portion of the heparin comes from the liver. Weil (140) demonstrated that if antigen is added to the blood

of a sensitized dog no change in coagulability occurs, but if this blood is then perfused through the liver from that animal it will then become incoagulable. Similarly various observers have reported that the injection of antigen into a sensitized dog whose liver has been extirpated or shunted out of the circulation, does not affect the coagulability of the blood.

Anaphylaxis in the guinea pig. First known as the Theobald Smith phenomenon, the anaphylactic reaction in the guinea pig was extensively studied by Otto (95, 96) and by Rosenau and Anderson (111). These investigators described the general character of the reaction and clearly defined the circumstances necessary for its occurrence. After the intravenous injection of antigen into a sensitized animal, there is a prodromal period of ½ to 3 minutes during which the animal usually sneezes, scratches its nose, becomes restless, discharges urine and feces, becomes weaker and lies down; acute respiratory difficulty then develops, and death from asphyxia may follow in 2 to 10 minutes. Intraperitoneal or subcutaneous injection of the antigen is much less certain, but may produce death with similar symptoms. The first analysis of the physiological mechanism involved was that of Auer and Lewis (9, 10). They demonstrated that the respiratory difficulty and asphyxia were due to a swiftly developing stenosis of the bronchioles and that in spite of violent respiratory efforts the animal was unable to effect any air exchange. They demonstrated that this bronchial reaction occurred in animals which had been curarized, whose vagi had been cut, or whose spinal cord, medulla and basal brain had been destroyed, indicating that the reaction occurred peripherally. The observations of Auer and Lewis have been confirmed by numerous workers and the demonstration by Dale (27) that the bronchospasm can be reproduced with the isolated, perfused lungs corroborated their conclusion that it was due to a direct action on the muscular walls of the bronchi and not due to a central or reflex nervous reaction. Auer and Lewis (9, 10), Anderson and Schultz (4), and Loewit (77) have recorded the systemic blood pressure in anaphylactic guinea pigs and report that there is an initial moderate rise followed by a gradual fall, such as occurs in asphyxia from any cause. It is thus generally accepted that the predominant reaction is pulmonary and that the circulatory effects are secondary in importance.

With the paramount reaction in the guinea pig clearly defined as a smooth muscle contraction, independent of nervous influences, it was but natural to look for smooth muscle stimulating toxins as important

factors in its genesis. Disregarding the various experiments purporting to prove the formation of an anaphylatoxin as the result of the mixing of antiserum and antigen, the first experimental search for such a substance in the intact anaphylactic guinea pig was, so far as the reviewer is aware, that of Hirschfeld and Hirschfeld (63) in 1912. These investigators tested the blood serum and plasma of anaphylactic guinea pigs by adding them to the fluid used to perfuse the vessels of the frog by the Trendelenburg technic. They reported that a vaso-contracting substance was found that was apparently liberated during the shock reaction. They commented upon the vagaries of its appearance, upon the experimental difficulties involved in studying the guinea pig's blood during different phases of the shock reaction and suggested that similar experiments should be done on the dog. Apparently neither their experiments nor their suggestions were followed up. In 1926 Alexander, Holmes and Becke (3) reported some experiments that might be interpreted as implicating tissue anaphylatoxins of a smooth muscle stimulating character. They reported that when the antigen to which a guinea pig had been sensitized was ground in a mortar with the lung or liver of that animal and then added to the bath in which the uterus was suspended, in many instances they obtained a greater contraction than when the antigen alone was used. They offered no explanation of their results and they apparently did not realize the possible implications as they did not perform the obvious control experiments of mixing antigen with corresponding tissues from a normal animal and of testing both types of tissue-antigen mixtures on a normal smooth muscle preparation.

Watanabe (134) and Hosoya and Watanabe (65) approached the problem from the direction suggested by Dale's theory mentioned previously. They determined the amount of histamine-like substance in the lungs and other tissues of normal guinea pigs, of guinea pigs that had just received the first or sensitizing injection of antigen, of guinea pigs that had received the sensitizing injection about 12 days previously, of guinea pigs that had just received the second or shocking injection of antigen, and of guinea pigs that had been sensitized from 30 to 100 days previously. They reported that there were no significant changes in any tissues except the lungs. They reported that the lungs of guinea pigs that had been sensitized 12 days previously had a very high content of histamine while that of similar pigs that had just received the shocking injection of antigen was very much less. They refrained from concluding that this evidence indicated a release of histamine during

anaphylactic shock because they found similar low histamine values in the lungs of guinea pigs examined after the first injection which had not produced a reaction, and because they found that the guinea pigs which had been sensitized 30 to 100 days previously showed normal values of lung histamine although presumably they were just as sensitive as the 12 day animals. Rigler (107) previously had examined the pooled livers and the pooled lungs of normal guinea pigs for their content of histamine-like activity and compared the results with the corresponding tissues of guinea pigs that had been sensitized 14 days previously. He found no appreciable difference between the normal and the sensitized animals. Likewise Daly, Peat and Schild (32) failed in 1935 to confirm Watanabe's findings. Mackay (81), however, pointed out that the histamine equivalent content of guinea pigs' lungs was so variable (from 7 to 88 mgm. per kilogram of tissue) that definite evidence of either a rise or fall in lung histamine would be difficult to establish by these methods, unless very large numbers of animals were used.

In 1932, however, Bartosch, Feldberg and Nagel (13) approached the problem from a different angle. They used the isolated, perfused lungs of sensitized guinea pigs and found that a histamine-like substance was liberated into the perfusate after the addition of antigen to the perfusion fluid. The identification of the active substance as histamine was indicated in that both were methyl and ethyl alcohol soluble, both lowered the blood pressure of the atropinized cat and both stimulated the secretion of epinephrine by the adrenal glands. Wachstein (133), Daly, Peat and Schild (32), Ungar and Parrott (127), Ungar, Parrott and Levillain (128), and Schild (112) have confirmed these observations. Daly, Peat and Schild amplified the evidence that the active substance was histamine, and Schild furnished evidence that a number of tissues other than the lungs could also liberate the active substance upon the addition of antigen.

Simon and Staub (123) were apparently the first to detect increased quantities of histamine in the blood of intact guinea pigs. They used the assay method of Barsoum and Gaddum and employed guinea pigs that were sensitized to, and shocked by, arsenobenzene. This observation was confirmed by Code (24), using serum sensitized animals. He found an increase in the blood histamine of anaphylactic animals up to thirteen times the normal values. He also demonstrated that this increase was not due to the coincident anoxemia as normal animals allowed to breathe nitrogen showed no such changes. He estimated that if one could assume that the blood concentrations obtained were the

result of the discharged histamine being distributed in an amount of fluid represented by 60 per cent of the body weight, the amount of histamine apparently liberated was enough to approach fatal doses and therefore obviously of definite significance in the genesis of the symptoms. Actual identification of the active substance in guinea pig blood as histamine has not been made and is probably impossible because of the small amounts available. The chemical and pharmacological evidence is, however, so consistent and so extensive that it may be considered as adequate.

Went and Lissak (146) reported in 1936 that the isolated, perfused heart of the sensitized guinea pig shows a slowing and arrhythmia when antigen is added to the perfusion fluid. This effect was prevented by atropine and was not produced by histamine. As adding physostigmine to the perfusion fluid did not enhance the effect they assumed that choline itself and not an ester was probably concerned. In a subsequent study (147, 148) they reported that a determination of the choline content of the heart muscle before and after an anaphylactic reaction indicated a decrease in the amount of choline in the heart muscle and also that acetylation of the perfusion fluid obtained as before led to the formation of a substance having an acetylcholine type of action on the frog heart and on the leech. Martin and Went (86) likewise reported that the isolated heart of a sensitized animal is more sensitive to the effects of choline than is the heart of a normal animal, concentrations of 1 to 20,000 being effective in the former, while concentrations of 1 to 5,000 are necessary to produce effects in the latter. They concluded that there was both a liberation of choline during the anaphylactic reaction and an increased sensitivity to its effect. The evidence for the former appears to be better than for the latter, but as yet neither observation has been confirmed. The observations of Auer (7) on the protective effect of atropine against anaphylactic shock in the guinea pig might be considered to lend some support to the thesis that choline plays a contributory rôle to that performed by histamine. The extent of its participation in the anaphylactic reaction appears most likely, however, to be limited to the comparatively mild cardiac effects.

There is often comparatively little change in the coagulability of guinea pigs' blood during anaphylactic shock, although occasionally it may become incoagulable. It has not yet been determined whether heparin is concerned in this effect, but the presumption would be that it is.

Anaphylaxis in the rabbit. The first experimental study of anaphy-

laxis in the rabbit was made by Arthus (5) although Flexner (54) had witnessed and described the reaction considerably earlier. In this animal the anaphylactic reaction reveals itself either as a local or a general manifestation, depending upon the route of administration of the shocking dose of antigen. The local reaction was first described by Arthus and is referred to as the Arthus phenomenon. When the shocking injection is made subcutaneously there may result infiltration, edema, sterile abscess or gangrenous slough at the site of the injection. Some further reference to this reaction will be made later. When the reinjection of antigen is made intravenously the general reaction is seen. The respiration quickens, the animal sinks upon its abdomen, feces and urine are usually expelled, a fleeting hyperemia followed by anemia of the ears may be witnessed, the heart beat becomes feeble, agonal convulsions may develop and the animal may die within a few minutes. The carotid blood pressure falls greatly, this fall being sometimes preceded by a temporary rise. It is to be noted that the respiration does not become dyspneic as it does in the guinea pig and also that a very much smaller percentage of rabbits succumb than is the case for guinea pigs. Auer (8) and Scott (117) demonstrated that the bronchospasm occurring in the guinea pig did not develop in the rabbit. Auer noted the marked dilatation of the right heart and ascribed the circulatory effects to cardiac failure. Scott on the other hand, noted an obstruction to the flow of blood in the inferior vena cava and believed that capillary dilatation was a major factor in the circulatory effects. Airila (2), Coca (22), Drinker and Bronfenbrenner (48), and Grove (60) have reported convincing evidence that cardiac failure is not a primary factor but that it is secondary to a pronounced obstruction to the flow of blood through the pulmonary circuit. Coca found that during anaphylactic shock the rabbit's lung develops a greatly increased resistance to the passage of saline perfusion fluids. Since embolism and thrombosis can be excluded, he concluded that the increased resistance must be due to a spasm of the arterioles. That the same obstruction occurs in intact animals was inferred by Airila and by Drinker and Bronfonbrenner as they demonstrated a marked rise in the pulmonary artery pressure which persisted until cardiac failure supervened. Histological studies have shown that the pulmonary arterioles of the rabbit present a remarkable degree of muscular development, which appears to be consistent with this assumption, and it may be said that this explanation of the major manifestations of anaphylactic shock in the rabbit has met with general acceptance. More recently Abell and Schenk (1) have made

microscopic observations of the behavior of the smaller blood vessels and blood elements of the rabbit during anaphylactic reactions. They observed the vessels in the rabbit's ear, but there is no reason to doubt that similar reactions occur throughout the circulation. They noted that the injection of antigen provoked both a marked contraction of the arterioles, which in some instances would completely obliterate the lumina, and a marked stickiness of the leukocytes which resulted in clumps of cells adhering to the vascular endothelium and in some instances leukocytic emboli were formed which blocked the circulation in the capillaries and veins. This reaction is undoubtedly the explanation for the observation of Webb (137) who had called attention to the marked aggregation of leukocytes in the capillaries of the lungs of anaphylactic animals, as well as for the leukopenia of the peripheral blood. Dragstedt, Ramirez and Lawton (46) have recently reported that the perfusion of blood to which antigen has been added through the lungs of a sensitized rabbit results in a rapid reduction of leukocytes in the outflowing blood. It seems very likely that this leukocytic reaction may contribute to the effects of the arteriolar spasm in obstructing the flow of blood through the lungs.

That the circulatory reactions in the anaphylactic rabbit are consistent with the pharmacological effects of histamine was indicated by the observations of Dale and Laidlaw (31), Bally (11) and more recently by Rocha e Silva (109). Rose and Weil (110), however, reported that the blood histamine in rabbits is definitely reduced during the anaphylactic reaction rather than increased as it is in the dog and the guinea pig. This has been confirmed by Dragstedt, Ramirez and Lawton. At first glance this would appear to exclude histamine as a mediating agent of the reaction in this animal. Barsoum and Gaddum (12) and Code (23) have demonstrated that rabbit's blood normally contains an extraordinarily large amount of histamine and have presented evidence that the major portion of this is contained in the leukocytes. Assuming that the rabbit's blood, as a tissue rich in histamine, might react as does the histamine rich liver of the dog and the histamine rich lung of the guinea pig, Katz (68) has recently reported that when antigen is added to the blood of a sensitized rabbit in vitro, there is a striking shift of histamine from corpuscular elements to plasma. This has likewise been confirmed by Dragstedt, Ramirez and Lawton who reported in addition that the reduction in the blood histamine in an intact animal is associated with and probably due to the withdrawal of leukocytes from the circulating blood. The amount of histamine that

is liberated from cells to plasma is of such an order of magnitude that if the same reaction occurred in vivo as it does in vitro, it is justifiable to conclude that it is significant in the symptomatology in the rabbit. If the lungs of a sensitized rabbit are perfused with saline solution to which antigen is added there is apparently some histamine liberated from this tissue source (46, 69). It is, however, notably less than that obtained in similar experiments with guinea pigs, and in the intact animal is apparently insufficient to compensate for the histamine withdrawn from the blood by the imprisonment of the leukocytes. There is substantial reason, therefore, for believing that histamine does play a rôle in the anaphylactic reaction of the rabbit and that the fall in the blood histamine is the consequence of the reactions that have made its liberation possible and provided it with its opportunity to act.

There is evidence to indicate either that the leukocytes of the rabbit can be passively sensitized by antiserum without the necessity of any latent period, or that the leukocytes irrespective of any sensitization are injured by the reaction between circulating antibody and antigen. Dragstedt, Ramirez, Lawton and Youmans (47) have found that the simultaneous addition of antiserum and antibody to normal rabbit's blood leads to the same discharge of histamine from cells to plasma as occurs when antigen is added to the blood of a previously sensitized rabbit. They have also found that the simultaneous addition of antiserum and antigen to normal rabbit's blood, used to perfuse the lungs of a normal rabbit, leads to the same withdrawal of leukocytes and of histamine that occurs when antigen is added to the blood of a sensitized rabbit that is perfusing the lungs from that animal.

These observations seem to be of considerable significance in explaining some of the apparent discrepancies between the anaphylactic reaction in rabbits and that in the guinea pig and dog. For example, Friedemann (57) and Scott (117) both reported that no latent period is required for the passive sensitization of the rabbit. Similarly Scott and Opie and Furth (93, 94) have described a reversed anaphylaxis in the rabbit, i.e., a reaction occurring in an animal in which antigen has been injected before the antibody. There have been attempts to explain these reactions as being anaphylactoid in character, but this explanation is inadequate to explain why similar results do not occur in other animals with equal facility. Also it has been reported that the refractory or immune state can not be achieved in the rabbit. Guinea pigs and dogs that have been repeatedly injected with antigen may reach a condition such that the reinjection of antigen after a suitable interval does not provoke an

anaphylactic reaction. The common explanation given for this is based upon the experiments of Weil and of Dale and Laidlaw and is to the effect that an abundant circulating antibody can combine with and thus protect the fixed tissue cells from the antigen. The absence of a refractory state in the rabbit in spite of highly abundant circulating antibody is consistent with the view that the fixed tissue cells are of less importance than the circulating tissue cells in the genesis of the anaphylactic reaction in this animal. Finally the reviewer is impressed with the satisfactory explanation that these findings give to the experimental facts that the Arthus phenomenon is much more readily produced in the rabbit than in other laboratory animals. The subcutaneous injection of antigen into a sensitized animal, as is well known, leads to the aggregation of large numbers of leukocytes. These cells, richly laden as they are with histamine, by discharging more or less of their content can add the effects of this agent to those of the agglutinating cells per se.

Wenner and Buhrmester (145) have reported that acetylcholine can be detected in rabbit's heart blood during anaphylaxis, but Ratnoff (105) could not confirm their findings. No examination for choline has apparently been made, but the absence of much protective effect by atropine against the anaphylactic reaction in the rabbit suggests that it would have at most only a minor rôle.

Delayed coagulation of rabbit's blood is rather regularly seen in anaphylaxis, although the blood does not often become completely incoagulable. Eagle, Johnston and Ravdin (49) have demonstrated that such blood has a high antithrombic activity. If this is due to heparin as it is in the dog it would appear from an early observation of Auer (8) that this does not come entirely from the liver as he reported that the usual change in coagulability can be obtained in rabbits in which the liver has been shunted out of circulation.

Anaphylaxis in other animals. The anaphylactic reaction has been observed in a variety of animals but has not been studied extensively enough in any of them to permit of conclusions either as to the important physiological mechanisms responsible for the symptoms or to the question of whether tissue anaphylatoxins may play a rôle in the genesis of the reactions. Anaphylaxis has been studied in the cat by a number of workers (18, 48, 50, 82, 114). Cats appear to be rather difficult to sensitize and in addition many foreign proteins produce such marked effects without the necessity of a previous sensitization that there are some experimental difficulties involved in analyzing the reactions in these animals. They show a marked fall in systemic blood pressure,

with some rise in pulmonary artery pressure, analogous to the effects of histamine. The blood may become incoagulable. There is apparently less participation by the liver in the cat reaction than for the dog and less participation by pulmonary vessels in the cat than for the rabbit. No search for anaphylatoxins has been made in this animal.

Hanzlik, Butt and Stockton (61) have studied the anaphylactic reaction in pigeons and have described a contraction of the circular muscle of the crop associated with a relaxation of the longitudinal muscle, which effects they were able to duplicate with histamine.

Anaphylaxis in the rat has been studied by quite a number of investigators (53, 70, 78, 85, 97, 103, 116, 118, 125, 126, 148, 150) and there has been considerable dispute as to whether or not bona fide anaphylactic reactions can be induced in this animal. The rat is peculiarly tolerant to histamine as well as to anaphylaxis. There is evidence that adrenalectomy markedly reduces this tolerance to both. Suden (126) has shown that the systemic reaction in the rat (fall in blood pressure—mild uterine contraction) is similar to that produced by histamine. No direct evidence to implicate tissue anaphylatoxins has been presented.

Various workers have observed anaphylactic reactions in horses and cattle. In general the symptoms, dyspnea, salivation, edema, collapse, etc., are similar to the reported effects of histamine in these animals. Code and Hester (25), however, have reported a fall in the blood histamine of both the horse and the calf during anaphylactic shock. It may well be that some such mechanism accounts for this decline as has been described for the rabbit. In fact Code and Hester state that a centrifuged specimen of control horse blood yielded a well defined layer of white cells while a similar specimen of anaphylactic blood yielded but a thin film, suggesting the disappearance of the white blood cells during the reaction. Since their findings indicated that 65 to 85 per cent of the blood histamine is contained in the white blood cells, it seems logical to conclude that the decrease in blood histamine was associated with the leukopenia. It would therefore be interesting to determine whether the blood cells of the horse react as do those of the rabbit to the addition of antigen.

There is a considerable amount of literature which has not been cited that may be said to provide circumstantial support to the thesis that an anaphylatoxin of the nature of histamine is concerned in the anaphylactic reaction. It consists of reports purporting to prove that a number of immunologically non-specific substances such as anesthetics, adrenal cortex extract, thymoxyethyldiethylamine, atropine, epinephrine, etc.,

protect animals against anaphylactic reactions in about the same degree that they protect against histamine intoxication. While these reports are interesting and of considerable significance, it would carry this review too far afield to consider them here. Furthermore, with the anaphylactic reaction disclosed as a chain reaction (41), involving the antigen-antibody reaction, the discharge of anaphylatoxins, and the subsequent production of effects by these agents, it is apparent that the reaction may be modified or attenuated in a number of ways. Thus without the necessary proof of where or how a nonspecific agent acts it is impossible rightly to conclude from the mere fact that it does modify the reaction that it has done so by virtue of a specific antagonism to histamine, for example. Similarly there are a number of reports indicating that various substances such as glucose, sodium chloride, glycerine, calcium salts, etc., (see Hill and Martin, 62) may modify or prevent the anaphylactic reaction while they do not prevent the effects of histamine. It is equally invalid to conclude from such evidence that histamine is of no consequence in the anaphylactic reaction.

It seems appropriate, however, to mention and discuss briefly some of the arguments which have been raised from time to time to challenge the relation of histamine to the anaphylactic reaction. One of the oldest arguments apparently arose from the observation of Lura (80) that sensitized guinea pigs which have become desensitized or anti-anaphylactic to the antigen do not show any desensitization to histamine or that an injection of histamine does not desensitize the animal to the antigen. However, as Wells (144) has pointed out, we would not expect histamine to desensitize if it is the product of the antigen-antibody reaction, since it is this reaction alone that is prevented by desensitization. Smith (124) has reported that quinine augments the susceptibility of sensitized animals to the injection of antigen but does not increase their susceptibility to histamine. This observation, which has not been confirmed to the reviewer's knowledge, might be due, as Wells suggested, to an action that quinine may have upon the antigen-antibody reaction. It certainly presents no serious contradiction to the evidence for the rôle of histamine. One of the commonest challenges to the histamine pathogenesis has been that it failed to explain one of the dominant symptoms, namely, the incoagulability of the blood. As has been discussed, this manifestation is due to another tissue anaphylatoxin, heparin, which may or may not come from the same source as the histamine and may or may not be discharged in parallel amounts. It is obvious that histamine and heparin explain only those symptoms which they do

explain and that such items in the category of symptoms which they fail to account for demand their respective explanations but do not invalidate in any way the part that these agents play. Another argument against the participation of histamine was that histamine does not duplicate the vascular reaction of anaphylaxis in the rabbit. The effects of histamine on the blood pressure of the rabbit vary with the dose as well as with the anesthetic and Rocha e Silva (109) has shown that if anaphylactic reactions of varying degrees of severity are compared with the effects of varying doses of histamine, the conclusion that the one bears the imprint of the other becomes strengthened rather than nullified. Kellaway (70) believed that the isolated uterus of the sensitized rat, since it contracted upon the addition of antigen while it relaxed upon the addition of histamine, contradicted the histamine hypothesis in anaphylactic reactions. Suden (126) has answered this by demonstrating that the reaction of the rat uterus is different in situ from what it is in the bath, and that the reaction in the intact animal in anaphylaxis is similar to that produced by histamine. It should also be added that the addition of antigen to the isolated uterus in a bath should not be expected to produce a histamine effect unless it could and did liberate histamine from the uterus. That antigen can liberate histamine from a large variety of tissues does not prove that it can do so from all tissues and it may well be that the rat uterus in situ reacts to histamine which has been liberated from other tissues, while the corresponding uterus in a bath shows only the non-specific effect of a foreign serum. The isolated plain muscle from a sensitized dog gives no significant response to the specific antigen, yet the plain muscle of the dog is sensitive to histamine and, in addition, a contraction of smooth muscle organs occurs during the anaphylactic reaction in the intact animal.

SUMMARY

The evidence may be considered conclusive that a tissue liberation of histamine, of heparin, and possibly of choline occurs during the anaphylactic reaction in various animals. In the dog the liberation of heparin can completely account for the incoagulability of the blood and there is no reason to doubt that it may be found in other animals. There is no evidence that it is responsible for any of the other dominant symptoms as large amounts of heparin can be injected into animals without harm. The liberation of histamine can account for practically all of the other effects and there is no well defined prominent symptom demanding an alternative explanation. Such changes as loss of complement, etc.,

are excluded from this statement and may be considered as being related to the antigen-antibody reaction per se. The observations of Went suggest that choline may participate in the reaction in the guinea pig and possibly of course in other animals, although further evidence is necessary to establish this. The leukopenia in all animals can be explained by the mechanisms described by Abell and Schenk in the rabbit, and it is probable that leukocytic emboli may contribute more or less to the vascular reactions produced by histamine. For animals other than the dog, guinea pig and rabbit decisive evidence is lacking, but the symptomatology is nowhere incompatible with the conclusion that similar reactions take place.

The cardinal symptoms of anaphylaxis can thus be explained as being due, in the immediate instance, to an autointoxication by physiologically active substances normally resident in various tissue cells and liberated therefrom by some change in cellular permeability brought about by the antigen-antibody reaction. It is apparent that the tissues concerned may be either the fixed tissues as in the dog or guinea pig, or the circulating tissue as in the rabbit. It is also apparent that the reactions mediated by these anaphylatoxins may occur in the tissues from which they were liberated or in distant organs and tissues. The mechanism by which the antigen-antibody reaction leads to the rather special type of cell injury resulting in the discharge of the agents characteristic to the anaphylactic reaction presents the next challenge in the study of this interesting subject, but there is considerable satisfaction in being able to discard the various esoteric speculations regarding the pathogenesis of the symptoms.

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ANTIHORMONES

KENNETH WADE THOMPSON

Department of Surgery, Yale University School of Medicine, New Haven, Conn.

The present interest in the study of antihormones was precipitated by the experiments of Collip and Anderson (31a), whose foresight led them to study the sera of animals that had become refractory to the action of certain pituitary hormones. They discovered that the serum of an animal resistant to thyrotropic hormone prevented the action of this hormone in a test-animal. This effect was ascribed to what they termed an "*antihormone*", which apparently accumulated in the injected animal's serum in response to the hormone administered.

These results conceivably were what an immunologist would have predicted, for in the various texts of immunology there has accumulated evidence which indicates that specific cytotoxins or cell-destroying antibodies may be produced by the injection of extracts of specialized cells. More specifically Abderhalden (1) has described "Abwehrfermente" as appearing in the blood of experimental animals chronically treated with extracts of endocrine glands.

The discovery of the antagonistic action of sera of hormone-treated animals deservedly aroused much interest among endocrinologists and physicians. Surprisingly enough this degree of investigative enthusiasm apparently has not been shared by the immunologists, whose authorities indeed had predicted the discovery of antihormones. The work thus has not progressed as rapidly as one would desire, because, as will be seen later in this review, certain essential experiments dealing with the immunological aspects of the problem have not been undertaken.

Collip, in his original statements (27), likened the antihormone not to an antibody but to the "chalone" of Sharpey-Schäfer. This pioneer in endocrinology postulated (181) the existence of substances possessing purely inhibitory effects, and he proposed for them the term "chalone", based on a Greek word meaning "to relax". Collip (29) preferred to

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call the inhibitory principle "antihormone" rather than "chalone" on the following grounds:

> . . . In spite of the very logical nature of Schäfer's objection to the use of the word "hormone" to describe all of the internal secretions, both excitatory and inhibitory, the term has been accepted by general usage.
>
> While there may appear at first sight to be an analogy between the substances which I have called "antihormones" and the "chalones" of Schäfer, the theoretical conception of the mechanism of production and the physiological significance of the former were such as to demand the use of a descriptive and distinguishing term.

The reviewer believes that the term "*antihormone*" is the best and most descriptive name offered for the class of substances under consideration. The experimental evidence submitted thus far leads one to classify these inhibitory principles as immune bodies. There is ample precedent for the use of such a word as antihormone in this sense, where it would be comparable to antitoxin or antienzyme, all being antibodies that are produced in animals in response to antigens. The writer herein aims to use the word antihormone in a way that is consistent with immunological terminology, and in this respect differs from Collip whose tendency is to use the word in the sense of a chalone. For instance, Collip (29, 32a, b) has used the term "antithyrotropic hormone" while I have written "thyrotropic antihormone". It would seem permissible to use the term "antigonadotropin" to indicate the antihormone of a gonadotropic hormone, but I would not favor the term "antigonadotropic hormone". This discussion will serve to clarify the terminology used by the reviewer in the following paragraphs.

THE PROBLEM. The problem of the antihormones has resolved itself into the following parts: What is the nature of the inhibitory principles termed "antihormones"? How does the body produce them? What is their chemical nature? What are their physiological actions, their possible therapeutic uses? Although not all of these questions can be answered, nevertheless much information is available for this review. At first glance the material seems almost hopelessly confused by the number of hormones and the variance of their sources, the wide variety of species of test-animals experimented upon, and the number of different approaches used in the study of the problem. Much of the work is open to one serious criticism, which is that many of the investigators have been too broad in their claims, ascribing far-reaching conclusions to results that actually were narrow in their scope and application.

In order to simplify and to improve the readability of this review I have

commenced each of the longer sections with a summary of my interpretation of the published reports which then are quoted in greater detail. Not every publication has been listed for I had to be content with a selection of representative data on the various aspects of the problem. I have treated the antihormones as a group of substances of essentially similar nature, rather than as a number of separate entities each with special qualities. Should a supplement for this account be desired the reader is advised to consult a recent detailed review of the subject by Collip, Selye and Thomson (29, 32a, b), and briefer summaries by Evans (62), Foa (69), Marrian and Butler (127), Werner (209c), Wintersteiner and Smith (211), and others (70, 163).

Early reports. Prior to 1934, when the first publications on antihormones appeared from the Collip laboratory, there had been performed four types of experiments which have bearing on the present subject. The first dealt with an inhibitor for thyroglobulin, first found by Möbius (139) in the blood of thyroidectomized sheep. Such a preparation, called "antithyreoiden-Möbius", originally made available by the Merck Laboratory, has continued to be of experimental interest, especially in Europe, and will be discussed under the heading "antithyroid principles". Another group of studies dealt with the anti-insulin effects of various pancreatic preparations as shown by studies of the blood sugar values of treated animals. An anti-insulin action was attributed to the impure preparations of insulin. It is not clear from the experiments whether the effects on the glucose values were due directly to a specific chemical inhibitor in the extracts, or to some non-specific reaction of the injected animal such as the secretion of epinephrine. For this reason, these particular experiments and certain others contribute little to the problem save perhaps the knowledge that a concept of inhibitory factors or antihormone was present in the minds of these investigators: Cotte, 34; deJongh, 40; Meyer-Bisch and co-workers, 138; Nobel and Priesel, 142; Sayhun and Blatherwick, 179; Wichels and Lauber, 210; and others.

Investigations more pertinent to the present problem are those dealing with hormones as antigens. With few facts available concerning them antisera to the pituitary early received a fruitless clinical application in hypertension and diabetes at the hands of Legiardi-Laura (107, 108, 109). In 1923, however, Hektoen and his co-workers (90, 91, 92, 93a, b) reported the first of an important series of studies on the precipitin reactions of thyroglobulin. They demonstrated that an animal reacts to injections of thyroglobulin by producing a species-specific immune body (precipitin) and lesser quantities of non-species-specific immune

bodies. The precipitin reactions of pure thyroglobulin were successfully used by Hicks (98) to trace this substance in certain animal fluids. These investigations have been fortified by others with comparable results: Clutton *et al.*, 24, 25, 26; Picado and Rotter, 157, 160; Went *et al.*, 208.

Koyano (104) in 1923 injected ox-pituitary extracts into rabbits, with the stated aim of producing a pituitary antiserum. This preparation was injected into rats, whose adrenals and pituitaries finally showed cytological changes, which will be discussed later. Koyano unfortunately did not study the problem of the species-specificity of his antiserum. Somewhat similar experiments had been carried out by Masay (129, 130), who induced in animals a state akin to myxedema by the injection of a pituitary antiserum.

Despite the foregoing and other suggestions in the literature that crude glandular extracts when introduced parenterally might be antigenic, little heed was paid the fact that the resultant immune body might have physiological actions until the refractory state was observed in animals that had been injected for long periods with the available crude pituitary extracts. These observations came to be made in the following manner:

THE REFRACTORY STATE. P. E. Smith's publication (193), *The disabilities produced by hypophysectomy and their repair*, called forth a wave of contributions that established the existence of various "tropic" factors in the pituitary. An avenue was thought to have been opened for the possible reproduction of certain disease syndromes which conceivably could be attributed to an overactivity of one or another of the pituitary "tropic" hormones. Save for one exception (experimental acromegaly with the growth hormone) the long-term injection of pituitary extracts failed to produce the expected results when a heterologous pituitary preparation was injected into an animal. The injected animals developed a refractory state (6, 31a, 71, 97, 113, 117, 118, 119, 120, 121, 131, 133, 182, 185, 193, 203a, 218) whereas in P. E. Smith's original experiments with homologous preparations (i.e., rat pituitaries in rats) the hypophysectomized animals' subsidiary glands were maintained in a normal state.

DISCOVERY OF THE ANTISERA. Collip and Anderson (31a), while studying the nature of this sort of resistance in animals injected with thyrotropic hormone, noted that the sera protected test-animals from the therapeutic effects of injected thyrotropic hormone. An extract of liver and spleen from the thyrotropic-resistant rats also prevented the

rise in metabolism normally expected from thyrotropic hormone injections, whereas normal rat serum and horse serum had no such effect. Collip and Anderson stated that the thyroid glands of the rats showed the characteristic hypertrophy which normally is found following treatment with thyrotropic hormone alone, and further, that rats whose blood contained the inhibitory substance still responded with a rise in metabolic rate when fed desiccated thyroid. They suggested that these experiments indicated the possibility of a new method for the treatment of clinical hyperthyroidism.

Shortly thereafter other investigations (28, 29b, 30) in the same laboratory established the fact that similar inhibitory effects in several species of animals could be demonstrated for other principles, namely: the ketogenic (15a, b) and the gonadotropic hormones from the pituitary (188e), and for A.P.L. (9, 188d) (the anterior-pituitary-like hormone of the urine of pregnancy).[1] Collip stated that he had evidence for a growth antihormone (28). He also mentioned that similar antagonistic substances may occur spontaneously in the blood of certain patients; here no details were given, save to say that antagonistic substances in the blood of 10 patients occurred spontaneously and not following pituitary therapy.

Collip's antihormone theory. In order to explain the remarkable results of these experiments and to provide a working hypothesis for further experiments, Collip (27, 28) advanced his "antihormone theory", which may be quoted as follows: "For each hormone there may be an opposite or antagonistic principle. This antagonist is present in the normal subject but may not be detected until it exceeds in amount the hormonal substance with which it is balanced." This concept received further elaboration as a "*principle of inverse response*" where Collip postulated a system of hormonal check and balance of the endocrine glands, each hormone having its inhibitor.

THE METHOD FOR BIO-ASSAY. There have been a number of different methods used for the bio-assay of the antihormones. The principles followed in most of the reports have been similar to those initiated by Collip and Anderson (31a), viz.: 1, the antiserum is injected at a site different from that for the injection of the hormone; 2, a short period of pre-treatment with antiserum is followed by treatment with hormone while yet continuing the serum. The titer of antihormone activity

[1] The author hereafter for the sake of simplicity will use the abbreviation, "A.P.L.", in place of the following: anterior pituitary-like hormone of the urine of pregnant women; prolan, human chorionic hormone.

customarily has been estimated by determining the minimum dose of antiserum required to inactivate a standard dose of the hormone in the test-animal. Almost every species of the common small laboratory test-animals have been employed depending upon the type of experiment conducted. Zondek and his associates (224) report that rats are most suitable for testing the potency of antisera to A.P.L. It is important in these studies to make careful note of species of test-animal.

THE NATURE OF THE ANTIHORMONE. Collip (27, 28, 29, 32a, b) apparently rejected the notion that these inhibitory substances might be antibodies after he noted the immunological studies of Bachman (8), who wrote the following:

> A.P.L.-treated rabbits yield sera which give weak immunity reactions *in vitro* with solutions of A.P.L. and extracts of male urine, and stronger, but less constant reactions with human serum proteins. At the present time, however, there appears to be no clear connection between these phenomena and the A.P.L.-inhibitory property demonstrable in such sera with biological methods.

This statement of Bachman's cannot be interpreted to mean that the antihormone is not a variety of antibody, and it actually did not wipe away the suspicion of others that the immune processes of the body might play a rôle in the production of antihormones. There appeared, then, in this vein, a series of reports which gave further consideration to, and incidentally a certain amount of support for, an antigen-antibody concept of the antihormone.

THE NUMBER AND VARIETY OF ANTIHORMONES. The injection of pituitary *thyrotropic hormone* or the *gonadotropic hormones of the pituitary or of the chorionic tissues* readily induces in animals a refractory state associated with demonstrable antihormones in the serum. Most of our knowledge of these inhibitory substances is based on observations of the behavior of the thyrotropic and gonadotropic antihormones. However, under suitable conditions other pituitary hormones and certain nonprotein hormones give rise to effects which indicate the production of anti-hormones, but relatively little is known about them perhaps because tests for the hormones, which they inactivate, are uncertain of results or are more difficult to perform. Although the antihormones seem to be alike in their behavior, we must not assume that the properties of any one of them are the properties of the group until this has been demonstrated.

Mention should be made of the fact that in addition to the antihormones which have physiological effects, the hormonal preparations may

also give rise to antibodies that have no demonstrable physiological effects (11, 12, 13a, b, 190). Certain other principles which are not antihormones but which may be confused with them will be discussed under the title "anti-thyroid principles". Engel (57, 58, 59) has suggested that the gonadotropic inhibitory principle may be a product of the pineal gland, but what little evidence he has offered has not been supported. Since the thyrotropic and gonadotropic antihormones will receive much consideration later, now a brief description of the other antihormones will serve to present the knowledge which is available concerning them.

Collip (28) assumed that the *growth-hormone* may give rise to an antihormone, because hypophysectomized rats treated with a highly purified preparation failed to maintain satisfactory growth despite continued treatment. Evans *et al.* (61) confirmed these experiments of Collip's, but with crude extracts obtained excellent growth of similar animals. This and other experiences with the growth-hormone, especially the experimental reproduction of acromegaly, leads one to doubt whether an antihormone ordinarily is produced by the growth-hormone. However, final judgment on this and similar situations must be reserved until we are able to study the pure hormone under controlled conditions.

The *ketogenic principle* has a pronounced ability to induce the refractory state, and the antiserum confers similar resistance on test-animals. The refractory animal's own ketogenic hormones may be inactivated (15a, b). Likewise, animals develop a refractory state to the pituitary *adrenotropic hormone* (203a), but little is known about this antihormone.

Prolactin preparations also call forth antihormones. Young's (214) summary of his own work here will be quoted, for it may have significance for the thyrotropic hormone as well as for prolactin; viz.:

> 1. Large doses of ox prolactin possessing no detectable thyrotropic activity were administered daily to rabbits and monkeys for long periods. The serum of animals so injected for 12 weeks or more possessed marked ability to prevent the hypertrophic response of the pigeon crop gland to prolactin. 2. The average weight of the young reared by lactating mice which received daily injections of rabbit antiserum was slightly, but probably significantly, less than that of the young of control animals receiving similar injections of normal serum. This was possibly due to partial inhibition of milk secretion in those animals receiving antiserum. 3. Rabbits which had received daily injections of prolactin for 18 weeks exhibited a normal hypoglycaemic response to insulin after the administration of a potent pituitary glycotropic preparation, i.e., they were insensitive to the glycotropic action of the extract. No evidence was found, however, that this resistance to the glycotropic action of pituitary preparations was transmissible to normal animals by the serum of the resistant rabbits. 4. Although the prolactin

used to prepare the sera contained no detectable thyrotropic activity, the antiserum obtained from the rabbits possessed substantial antithyrotropic activity. By thyrotropic activity is meant the ability to induce hypertrophy of the thyroid gland of the immature guinea pig.

The essentials of these experiments have been confirmed by Rowlands and Young (177), and by Bischoff and Lyons (14). Prolactin, apparently, can be precipitated from an extract by incubation with prolactin antiserum, thereby reducing the physiological activity of the extract (Strangeways, 196). This method conceivably might be useful in the purification of extracts.

An antihormone for the *diabetogenic hormone* has been considered as the explanation for results of Dohan and Lukens (43), who injected pancreatectomized dogs with crude anterior pituitary extracts and after a time observed a decrease in the glycosuria. The animals in some respects came to resemble Houssay preparations, and the possibility was entertained that the diabetogenic principle was inactivated by the production of an antihormone in the animals.

Certain *non-protein hormones* have given rise to a state of refractoriness with anti-substances in the serum. Hartman and his associates (89a) have observed that *adrenal-cortical hormone* preparations lost their effect after repeated injections into normal dogs. The serum of dogs resistant to the adrenal-cortical hormone, when injected into other dogs, was found by Toby and Lewis (204) to inhibit the effect of cortin. These antigenic cortical extracts were highly purified, contained very little solid material, were free from protein, and contained practically no nitrogen. These extracts gave precipitin reactions with serum from the refractory but not from normal dogs. There was some evidence which indicated that the antisera had some power to fix complement. More recently Hartman *et al.* (89b, c) determined that crystalline corticosterone itself did not produce the inhibitory effect, but this did occur when the pure product was combined with swine serum before injection. This use of swine serum is a well known "trick" of immunologists whereby a substance not ordinarily antigenic is rendered so by conjugation with an element in the swine serum; the combined substance reacts as the antigen. The substance which confers the specificity but not the antigenicity is termed "*haptene.*" Sulman (197a), as will be seen later, claims that A.P.L. is not a haptene. Swine serum is not the only agent that confers antigenicity on substances which are not antigenic. For example, phenol has been used in this manner to produce antigenic adrenaline (199).

Brandt and Goldhammer (19) have produced complement-fixing antibodies to an *androgen* and an *estrogen* by mixing these substances with swine serum before the injection. In their experiments, however, the hormones were not inactivated physiologically by the antisera, whereas such was the case in Hartman's experiments with preparations of cortin. The injection of estrin alone apparently does not call forth antihormones (37), although this and other sex-hormone substances exert on the gonads and other tissues and hormones an inhibitory effect that might be confused with an antihormone effect (38, 72, 74, 152, 164).

Somewhat different, however, is the well known refractoriness to the repeated administration of the *parathyroid hormone*, for Taylor *et al.* (200) have presented proof that it is not due to an anti-substance in the serum. The sera of their resistant animals failed to give complement-fixation reactions with the extract or to inhibit the action of parathyroid extract biologically. The refractory animals tolerated massive doses of irradiated ergosterol that normally would be toxic in dogs not so treated with the parathyroid extract. Somewhat akin to parathyroid refractoriness is the state Selye (187) has observed resulting from prolonged treatment with estrogen. The refractory state was not transmitted by the serum of the animal.

THE NATURE OF ANTIHORMONES. *1. Hormonal preparations interpreted as antigens.* The foregoing account has described how the antihormones were presented as agents having important physiological actions. At once there was discussion as to their nature, and Collip and his associates proposed that they be looked on as chalones rather than as immune bodies, but he named them "antihormones." Successive reports appeared amply confirming the fact that there are such inhibitory substances, but each report tended to place them in the class of substances called antibodies rather than in the class called chalones.

Lacking pure hormones or pure antihormones the majority of investigators have had to be content to perform experiments devised to show the pattern of antihormone behavior; the facts were then compared with what we know about antigens and antibodies or hormones and chalones. In every important respect antihormones were similar to antibodies. The problem is made doubly difficult by the fact that we do not know the physical or chemical nature of the immune bodies, of which naturally there are a great number. Furthermore, the antihormone may be a variety of antibody that hitherto had been unknown and for which we have no precedents, save those common to antibodies in general.

Although the antihormones tend to appear and to disappear with the precipitins and complement-fixing antibodies, there has been no success in attempts to correlate more definitely these antibodies with the antihormones. Indeed the evidence clearly indicates that the antihormones are not linked with the above types of antibodies. On the basis of the observation that A.P.L. reacts with its antihormone quantitatively rather than by a process of adsorption, the suggestion has been made that this antihormone is a special type of ferment, and not a true immune body. One should not hastily use this evidence because the immunologist probably would tell us that certain immune bodies do react quantitatively with their antigens.

In several other respects the antihormones have behaved as if they were antibodies, viz.: they are not called forth by homologous extracts and do not appear in parabiotic animals with prolonged pituitary hormonal hyperactivity. Neither do they appear when a crystalline hormone (corticosterone) or a highly purified preparation is used. Nor do they appear in animals when the reticulo-endothelial system (the site of origin of immune bodies) is thrown out of function, or is inactive, as it is in the young or in certain states of debility. The antihormones seem to have a humoral action; no fixed tissue, neither the affected subsidiary glands nor the one originating the inactivated hormone, takes a necessary part, save the reticulo-endothelial system which produces the antihormones. Likewise, the changes in species-specificity of antihormones proceed on lines that have precedents in immunology. The finer details of the non-species-specificity of antihormones and the possible bearing of the Forssman or heterophile antigen upon this problem await special study.

One inevitably concludes from the present evidence that the antihormones belong to the class of substances called immune bodies, but they are a variety that has certain unusual characteristics. The hormonal preparations that stimulate their production are therefore antigens. These antigens evidently belong to the group that immunologists term "*complex antigens*," for the hormone can be separated by suitable methods from the carrier substance, which is antigenic while the hormonal radical is not. Although there exist data indicating that the hormonal portion of the antigen is a haptene, yet at present this cannot be assured; the various hormones may differ in this respect. The data now follow:

Twombly (205) demonstrated that preparations of A.P.L., which had been almost completely inactivated by heat or by ageing, were as

efficient in producing an antihormone as were active preparations. In Twombly's experiments, the antihormone titer increased with the precipitin titer (206). The foregoing experiments were confirmed by de Fremery and Scheygrond (39), who also noted that the serum of non-pregnant mares did not produce antihormone effects when injected into rabbits. Zondek *et al.* (219), however, claim that the A.P.L. is not completely inactivated by boiling, and that the antihormone-producing activity is due to the residue of active hormone.

Werner (209a, b, c) was able to induce in male guinea pigs a refractory state to a thyrotropic preparation made by a sodium sulphate precipitation method from bovine pituitaries. This he was unable to do with an extract equally potent in thyrotropic activity made of bovine preparations by flavianic acid precipitation. The foregoing work of Werner has been confirmed by Cutting *et al.* (35). Werner also showed that the thyroids of the refractory guinea pigs were stimulated by the flavianic acid product, and further, that the serum of non-refractory guinea pigs that had been injected with the flavianic acid preparation had no antihormone effect in test-animals. These experiments were interpreted to mean that the antihormone was produced by a substance associated with the hormone, but not necessarily by the hormone itself.

Eichbaum and associates (49) studied the problem of the antigenic properties of hormone preparations, and came to the conclusion that the complement-fixing antibodies produced by the injection of urinary extracts were due to a urine antigen, and not to the A.P.L. in the urine injected. They found that the antibodies for urine antigens did not react with antigens from homologous blood or skeletal muscle. In another experiment (50) they injected male and female rabbits with A.P.L. and observed in serum, first, precipitin and complement-fixing antibodies, which had no neutralizing effect upon A.P.L.; next, biologically active antihormones appeared; and lastly, after continued injections the precipitin and complement-fixing antibodies disappeared, leaving unimpaired in the serum the inhibitory action. These facts were interpreted to indicate that the antihormone activity is not necessarily affected by the precipitin or complement-fixing reactions. Brandt and Goldhammer (18) have come to similar conclusions.

Somewhat related experiments are those of Gegerson *et al.* (73), who prepared antisera in rabbits by the injection of A.P.L. and of bovine pituitary extracts, and then incubated the antihormone sera with human and bovine serum respectively. The heavy precipitate was removed, but the remaining serum retained its antihormone activity. This indi-

cated that the antihormone is a specific substance unrelated antigenically to the serum proteins. Gustus, Meyer and Dingle (85) also working with precipitin titers were unable to correlate precipitin titer and antihormone activity when they used monkey antiserum to pregnant mare gonadotropic hormones. A number of investigators (41, 42, 135, 153, 155, 159) were unable to correlate the precipitin titer with the antihormone activity of the sera, although some others found evidence that appeared to connect the two (60). Meyer and Wolfe (137), using gonadotropic hormone of pregnant mares, have shown that monkeys produce precipitins and antihormones following injections of small quantities of hormone, but that the precipitins are not responsible for the antigonadotropic action of the sera. Gordon *et al.* (79) used highly purified preparations of the mare chorionic hormone which produced an inhibitory action but no precipitins. The antihormone-producing property of the extract was possibly enhanced rather than decreased by the process of purification (78, 79).

Sulman (197a) has carried out numerous experiments attempting in one particular report to answer the question "does the gonadotropic hormone (A.P.L.)[2] induce antibodies or antihormones?" The claim was made that in rabbits "pure" A.P.L. did not call forth complement-fixing antibodies, whereas crude A.P.L. did do so. "Pure" A.P.L. coupled with swine serum also called forth complement-fixing antibodies, which, however, reacted only with the mixture and not with the A.P.L. alone. From these facts Sulman concluded that the "pure" A.P.L. was neither an antigen nor a haptene, but was some unusual form of protective ferment. *The "pure" prolan of Sulman was prepared by a pharmaceutical firm, and Sulman gives no references concerning the method for preparing the product, and no proof of the purity of the substance.*

Van den Ende (207) has submitted a comprehensive report and discussion on the preciptins in antigonadotropic sera, giving the following summary:

1. Antisera to an active gonadotropic extract of pregnancy urine contain precipitins demonstrable with a variety of urinary extracts, independent of their hormone activity. The amounts of precipitins present could be determined by optimal proportions. 2. Precipitin content and antigonadotropic power showed no constant relationship to each other. 3. Pregnancy urine extracts contain multiple antigens. 4. Absorption of precipitins by an extract of the urine of males resulted also in the removal of all precipitins for urinary gonadotropic extracts, but only partly reduced the biological inhibitory power. On the other

[2] The insert enclosed in parentheses is the reviewer's.

hand, absorption of precipitins for human serum only slightly reduced the precipitins for urinary extracts, and left the biological inhibitory power completely unimpaired.

Although mention of thyrotropic antihormone has been very brief in the foregoing listing of experiments it has received comparable investigative consideration, and the findings, save those of Loeser and his associates, are similar to those obtained with the gonadotropic antihormone. Further data where the behavior of the antihormones was compatible with that of immune bodies are the following: Okkels (144) using preparations of whole organs has observed that the thyroid glands of animals refractory to thyrotropic hormones are *not in themselves refractory but can be restimulated*. Thyroids from rabbits made resistant to an alkaline extract of bovine hypophyses were perfused in the Lindberg pump with fluid containing the extract to which the animal had become refractory. The thyroid responded as readily as control glands from untreated rabbits.

Perhaps the most interesting contribution is that of Gordon, Kleinberg and Charipper (75, 76, 77), who demonstrated that *the site of production of antihormones is the same as that of immune bodies, namely, the reticulo-endothelial system.* After a splenectomy or a blockade of the reticulo-endothelial system by the injection of trypan blue, antihormones to A.P.L. appeared one or two weeks later than in controls. Guercio and Cazzola's (80, 81) observations led them to believe that splenectomy merely inhibits the formation of precipitins but not the antihormones. Collip and Anderson (31a) found that saline extracts of liver and spleen from thyrotropic hormone resistant rats had an antihormone action.

Although the reticulo-endothelial system appears to be necessary for the formation of antihormones, the endocrine glands do not appear to be so vital. Collip and associates (9, 31b) have reported that the hypophysis is not necessary nor is the ovary, and this was confirmed by others (82, 83, 123). Sulman (197a), and Brandt and Goldhammer (18) found that castration in no way impaired the ability of rabbits to produce antihormone to A.P.L. Chou (23), Oudet (145, 146, 147), and others (114, 116) have observed that thyroidectomized rabbits produced antihormones to thyrotropic hormone, whereas Eitel and Loeser (56) and Loeser (124) on the other hand, observed that the antithyrotropic action disappeared from the serum of one wether 20 days after thyroidectomy. Hartman *et al.* (89b) observed that adrenalectomy had no effect on the development of the refractory state to cortin.

2. Specificity of antihormones. The first experiments with anti-

hormones were interpreted to indicate that the refractory state was limited to the preparation with which the animal had previously been injected (9). It was soon appreciated, however, that certain refractory animals showed signs of inactivation of their own pituitary hormones. This obviously indicated a loss of the species-specific quality of the inhibitory substance. Black, Collip and Thomson (15b) had shown that the characteristic acetonuria of fasting was diminished in resistant animals following repeated injections of a ketogenic extract, and Black (15a) observed that the ketogenic hormone-resistant animal developed practically no ketosis when given massive doses of phlorhizin, despite the fact that the usual glycosuria was produced.

Many investigators (73, 136, 148, 167, 171, 202b, 203b, 212, 213, 220) later conclusively demonstrated that there is a considerable crossing of the inhibitory properties when the antisera are produced in one animal species by long term injections with hormonal extracts of other species. These observations may be compared to those in another immunological situation devised by Stokinger and Heidelberger (195) where species-crossing antibodies to purified thyroglobulin were detected with the aid of the precipitin reaction. Kestner (102, 103), using an extract of the thyroid glands of swine, produced an antiserum in sheep which depressed for several weeks the basal metabolic rate of rabbits, dogs and rats. Oudet (149) has produced a thyrotropic antiserum by injecting rabbits with an extract of bovine muscle. The antiserum inactivated thyrotropic hormones from oxen, but not from sheep.

The non-species-specific effect of antihormones has not always been produced by heterologous extracts, as is illustrated by the early work already mentioned and by the following experiments: Fluhmann (67, 68) injected rats for approximately 100 days with a gonadotropic extract of acetone-dried human pituitaries, and observed that the pooled antiserum inactivated the gonadotropic action of human pituitaries and of A.P.L., but did not inactivate a similar action of sheep pituitaries. He also injected rabbits for 74 days with a gonadotropic extract of human pregnancy blood. The final antiserum tested was specific in inactivating the extract injected, but had no effect on gonadotropic extracts of human or sheep pituitary glands. Cope (33) found that bovine pituitary antiserum from rabbits did not inactivate human pituitary thyrotropic hormone. Rowlands and Spence (176) found antihormones in the sera of boys treated with pregnant mare serum, and their inhibitory action was specific for the extract injected, having no effect on human pituitary gonadotropic hormone. Meyer and Gustus (135) injected immature

monkeys with pregnant mares' serum and found the antagonistic effect was highly specific in the antiserum, there being no inhibitory effect toward the hormones of A.P.L., sheep or human pituitaries, when tested in immature rats. Yasuda (212) injected rabbits with A.P.L. and found that the resultant A.P.L. antiserum did not inactivate bovine gonadotropic hormone when tested in rabbits. Sulman (197a) found that antihormone against A.P.L. in rabbits had no inhibitory effect on the gonadotropic hormone of the serum of pregnant mares. Zondek *et al.* (220) report that the antihormone to A.P.L. in rabbits is highly specific; less than 0.5 per cent of the effectiveness being available for a heterologous preparation, whereas 7 per cent of the activity was available for a homologous factor (serum of pregnant women), and the remainder for the specific factor.

On the problem of organ-specificity or of hormone-specificity there is less information available. There appears to be no doubt that one can prepare with the proper treatment an antiserum which inactivates the gonadotropic action of chorionic as well as pituitary hormones. For example, Thompson and Cushing (203b) injected a dog for many months with a sheep pituitary preparation, and the dog's serum inactivated the gonadotropic effects of several species of pituitary glands as well as the action of pregnant mare serum and A.P.L. Likewise a dog injected with pregnancy mare serum inactivated gonadotropic extracts from sheep pituitary glands. Rowlands and Parkes (175b) injected a goat with A.P.L. and found that its serum inactivated the gonadotropic hormone of human pituitary glands. In regard to class-specificity, Parkes and Rowlands (151) have shown that in birds antisera to mammalian thyrotropic and gonadotropic substances are ineffective.

Work on the hormone-specificity of antihormones is hampered by the lack of chemically pure pituitary hormones. One of the few experiments with so-called "pure preparations" is that of Rowlands and Young (177) who found that prolactin antisera, produced by injection of an extract apparently free of thyrotropic activity, had a markedly anti-thyrotropic effect. Here however, there remains the suspicion that a trace of thyrotropic hormone may have been present in the antigenic extract. With the available pure non-pituitary hormones there seems to be no doubt of a strict hormone specificity, when antihormones are successfully produced.

Antihormones for gonadotropic (176) or thyrotropic (194b) hormones apparently develop in man, as they do in other animals, under appro-

priate conditions. Thus far in patients injections have been terminated when the refractory state was noted, and no instances of inactivation of the subject's own pituitary hormones have been recorded. Whether these antihormones are the same as the inhibitory substances which occasionally are found in normal human sera has not yet been determined.

3. Experiments with homologous preparations. The data quite clearly indicate that the injection of homologous preparations in animals does not ordinarily give rise to antihormone formation. Whenever the antihormone effect has been obtained with these conditions, the antisera have been weaker in potency than antisera resulting from heterologous preparations. These few exceptions could conceivably be explained by assuming that some of the proteins were denatured in the process of preparing them for injection. This might render them antigenic in the homologous species. The immunologist conceivably might offer another more suitable explanation for these exceptional observations.

The failure of homologous preparations ordinarily to stimulate the formation of antihormones lends strong support to the supposition that the antihormone is a variety of immune body. Certain cleverly designed experiments with parabiotic rats have conclusively proved that an over-stimulation with pituitary hormones can proceed for long periods without any suggestion of the development of the refractory state, or of antihormones. These experiments strongly oppose the chalone concept of the "theory of inverse response." The following will serve to present the material available on the subject of homologous preparations:

Twombly (205, 206) and others (44, 193) were unable to demonstrate antihormone effects in human sera after prolonged parenteral treatment of patients with A.P.L. (human, in origin). Likewise, Rowlands (168) and Thompson (201) were unable to produce gonadotropic antihormones in sheep injected with crude extracts of sheep pituitary glands. These investigators observed a slight augmenting effect in some of their tested sera. Collip (30), on the other hand, noted a weak antihormone effect in the serum of two out of four sheep injected for long periods with a crude, gonadotropic extract of sheep pituitaries. The antisera in these particular animals of Collip's were effective against bovine pituitary, pregnant mares' serum, and possibly hog pituitary extracts. Collip also observed some signs of synergism between some of the sera and the gonadotropic substances.

Supporting P. E. Smith's original contribution, Katzman, Wade and Doisy (101a) were unable to demonstrate refractoriness in female rats

following the daily administration of one or two rat pituitaries for a period of from seven to nine months. The serum of these animals gave no gonadotropic or thyrotropic antihormone effect in test rats or guinea pigs, but did give an augmenting effect. On the other hand, Selye, Collip and Thomson (188e) found that the ovaries of rats receiving daily implants of rat pituitaries for 68 days were normal or subnormal in weight; this fact they interpreted as evidence of a refractory state. Anderson and Evans (4) have claimed that large doses of rat thyrotropic hormone injected into rats gave rise to a thyrotropic anti-substance in the serum of the animals; the effect was detected by a slight depressant action of the serum upon the O_2 consumption of guinea pigs receiving a standard dose of thyrotropic hormone.

Even more satisfactory experiments with a *more physiological type of homologous administration of hormones have been those using parabiotic rats* (36, 46, 128, 132). These ingenious studies showed that the gonadectomized parabion caused a continued stimulation of the gonads of the normal or hypophysectomized twin, producing in it a constant state of estrus as a result of the greatly increased output of pituitary gonadotropic hormone from the castrated animal. This condition of continued stimulation was maintained for long periods of time by different methods, and indicated that under these conditions no gonadotropic antihormone was produced in either animal in response to the pituitary hypersecretion. Kupperman *et al.* (105) showed that an antihormone in rabbit serum prevented the action of endogenous gonadotropic hormone secreted by the pituitary gland of a castrated rat in parabiosis with a normal female.

4. Time of appearance and disappearance of antihormones. The antihormone production begins in an animal shortly after the first injection of a suitable preparation, and the inhibitory substance accumulates in sufficient quantities in the blood stream to produce a noticeable effect in 7 to 12 days. This effect is the inactivation of the injected extract, producing the refractory state. After 21 to 28 days the serum is highly active in its power to inactivate the antigenic extract in test-animals. After long periods of injections into suitable animals the non-species-specific antihormones appear in sufficient quantities to inactivate the injected animal's own hormones, and at this time the antiserum can be expected to have actions on antigens from species other than the one used for the injections.

After injections of the antigen are stopped the antihormone activity gradually decreases in the serum, so that usually it may not be detected

after 30 days. In one instance it was detected for 2 months after the injections were stopped. A single injection of antihormone in homologous serum may protect an animal from the antigenic hormone for from 8 to as many as 30 days. Mature animals seem to produce antihormones more effectively than young ones; rabbits possibly are more satisfactory antihormone-producers than are other animals. Crude hormonal preparations are more effective in calling forth antihormones than are purified hormones. The following data are available:

There is ample evidence quoted earlier which indicates that the antihormone accumulates in sufficient quantity to induce the refractory state (96, 99) within two to four weeks after injections are started. Indeed, Hertz and Kranes (97) observed a regression of bovine thyrotropic activity in rabbits after 7 to 12 days of injections. Hamburger (87), using rabbits, observed that an antihormone to the mare serum gonadotropic hormone could be detected as early as the 9th or 10th day after treatment was begun. The time depends apparently upon the species and nature of the animal injected and the amount and quality of the preparation injected.

Simonnet (191) was able with one injection of rabbit antiserum to A.P.L. to protect a rabbit from the action of this hormone for as long as 3 weeks. When the hormone was administered first the antiserum would still check the hormone action if given within 24 hours. Rowlands (172, 173), who has made extensive observations of specificity of antihormones studied the properties of sera of rabbits injected with bovine pituitaries, and observed that these sera inactivated the luteinizing effect of gelding pituitaries. This particular power of the serum increased up to 7 weeks, was then constant for 2 weeks, and thereafter declined somewhat, although the same injections were continued in the donor rabbits. Rowlands and Spence (176), who produced antihormone to pregnant mares' serum gonadotropic hormone in boys, found that the titer in the serum of two patients rose for 3 weeks after the treatment was discontinued. Meyer and Gustus (135) daily injected immature monkeys with 5 R. U. of the gonadotropic substance of pregnant mares' serum; the antihormone was detectable as early as the 27th day, was highly potent at the 39th day, and in some animals persisted 67 days after the injections were stopped.

Zondek and Sulman (221) observed that while the antihormone in rabbit serum had a definite inhibitory effect upon A.P.L. when the two substances were injected separately into the test-animals, yet the inhibitory effect was stronger if the substances were allowed to interact

by mixing and incubating them before the injection. The anti-gonadotropic effect persisted for 8 days after the injection of a single dose. The antihormone circulated in the blood of the test-animals for at least 60 hours after a subcutaneous injection. Eitel and Loeser (55), studying the thyrotropic antihormone in sheep, observed that the titer of the antihormone increased to a maximum at the 4th to 5th week, and then declined, vanishing after 9 to 12 weeks despite continued treatment. The formation of gonadotropic antihormone is said by Zeldenrust (216) to be equally efficient in male or in female rabbits. Mature rats are said to produce more antihormone in response to A.P.L. than do immature rats (78). Ascorbic acid treatment does not enhance the production of antihormones (Zeldenrust, 217). The use of crude extracts (Rowlands and Young, 177) or of glands which have been stored for a period of time (Cutting *et al.*, 35) seems to increase the antihormone-producing power.

5. Search for antihormones in sera of untreated animals. The sera of certain untreated animals have an inhibitory effect as if there were antihormones present. This antihormone-like action is perhaps more apt to be effective *versus* the thyrotropic than *versus* the gonadotropic hormone. The thyrotropic inhibitory effect is commonly found in the sera of sheep, but also it is found without any pattern of regularity in the sera of other animals. We do not know enough about these particular inhibitory principles to say whether or not they are the same as antihormones. The fact that normal sera have this inhibitory action has been cited as an argument in favor of the chalone concept, but so far as the writer is aware, the possible rôle of the heterophile antigen (162) in this respect remains to be investigated.

Collip and Anderson (31b) found gonadotropic inhibitory substances in the blood of ten normal patients, but were unable to demonstrate the presence of thyrotropic anti-factors in normal rats when hypophysectomized rats were used as test animals; likewise, they did not find such a principle in normal horse serum. Rowlands and Parkes (175a) found no anti-thyrotropic principle in the sera of normal cows, rabbits, or goats, but it was present to a slight degree in normal horse and sheep sera. Loeser and his associates (54, 55, 125) have found a thyrotropic inhibitory principle in normal ovine and canine blood, and Herold (95) has found a similar principle in guinea pig blood. When the inhibitory activity was present in the serum of untreated sheep Eitel and Loeser (53) were able to increase its titer by the injection of thyrotropic hormone.

Laroche and Simonnet (106) have observed that certain normal human sera contained an inhibitory substance for gonadotropic hormone and also an antibody (precipitin) to A.P.L. Scowen and Spence (186) found a slight antithyrotropic effect in serum of normal rabbits and in normal human serum, but no antithyrotropic effect was found in the serum from two patients with Graves' disease. Zondek and Sulman (223) were not able to find an antihormone of A.P.L. in the blood of pregnant women, nor in the blood of normal or amenorrheic or sterile women, nor in the blood of those suffering from tumors. Sulman (197a) found neither A.P.L. complement-fixing antibodies nor antihormone action in the blood of pregnant women in the 2nd and 4th months of pregnancy and during the first day or the first month postpartum.

Thompson (202b) has observed a thyrotropic inhibitory principle in the blood of two normal ewe lambs. This was not present in the sera of one normal mare, nor in six normal dogs. However, gonadotropic and thyrotropic inhibitory principles were observed in the serum of one human patient with the Cushing syndrome (Case: La Frank) but were not present in the sera of two supposedly normal patients (51). Other inhibitory effects have been obtained with sera from patients with carcinoma (165a, b) and with amenorrhea (198). Picado and Rotter (156, 157, 161) claim that normal human blood, especially of elderly or goitrous people, may have antithyrotropic actions.

Chou (23) stated that no inhibitory effect was found in the sera of five of seven guinea pigs that received normal rabbit serum, whereas the sera of two so treated showed a slight inhibitory action. He quoted Chen (22) of the same laboratory who had observed that the gonadotropic effect of pituitary in an immature rat was lessened by simultaneous injections of normal rabbit serum. They ascribed this antagonistic action of the serum to its toxic effect, which was manifested by a retarded growth-rate of the animals.

6. Augmenting effects of sera. The sera of animals that are undergoing treatment with pituitary extracts sometimes develop the power in test-animals to augment instead of to inhibit the action of gonadotropic extracts. This augmenting effect, sometimes called "progonadotropic", is entirely different from that produced by certain chemicals which act by altering the solubility of the hormone. The former is effective when the serum and hormone interact *in vivo*, whereas the latter must be mixed with the hormonal extracts *in vitro* before the injection. There is no exact knowledge of the nature of the augmenting principle, which has been observed with respect to only the gonadotropic hormone. It

appears to accompany the globulins of the serum. It might be a gonadotropic hormone retained in the animal sera, or another type of substance, possibly an antihormone to the antagonist. This problem deserves more investigation. The available data are listed as follows:

The augmenting principle has been produced in sera by injecting: 1, sheep pituitary extract into dogs or horses (202a); 2, sheep pituitary extract into sheep (30, 170a); 3, swine pituitary extract into a goat (170b); 4, horse pituitary extract or ovariectomized women's urine into rabbits (192). Sulman and Hochman (197b) observed the augmenting effect only once in all their experiments; in this one case the serum was obtained by the daily injection of a rabbit with 2 rat pituitaries for 5 months.

Katzman *et al.* (101a) and Thompson (202a) observed that the augmenting principle in serum stimulates the ovary of the immature rat. This naturally meant that the principle was not species-specific, and more important, it may have been a pituitary principle (110), possibly a follicle-stimulating hormone present in the tested serum. Thompson attempted to rule this out by observing the action of the augmenting principle in hypophysectomized immature rats. He found that it had no effect in these test-animals, whereas it stimulated the ovaries of normal immature rats. This point deserves further study by other investigators, for it appears to be an important method of differentiation. Thompson offered the tentative suggestion that the augmenting principle may be an antihormone to the antagonist (63, 86, 111, 112). Although Rowlands (170a) was inclined to agree with this interpretation, further information must be obtained to clarify the status of this principle, which should be regarded as a retained gonadotropic fraction until proved otherwise.

The augmenting principle is contained in the globulin fraction (202a), as are the antihormones and immune bodies and certain pituitary hormones. Katzman *et al.* (101b) observed that the principle was readily adsorbed by "Willstätter's type A" alumina from a globulin fraction but not from whole serum. It did not dialyze through a Visking membrane. The globulin precipitate could be frozen, desiccated, and then stored for long periods without loss of this activity.

There are other substances that have the power to augment the action of gonadotropic extracts. They are different from the above described principle because they must be mixed with the gonadotropic extract *before* injection, whereas the principle described above has pronounced effects when the mixture is *in vivo*. Examples of the sub-

stances requiring preliminary admixture with the extract for their enhancing properties are: 1, certain substances such as tannic acid (194) and the ions of copper and zinc (65) which produce relatively slowly acting proteinates of the hormonal preparation, and 2, certain elements of whole blood, namely, hemolyzed erythrocytes, hemoglobin, and heme; porphyrin, globin, and ferrous or ferric chloride do not have this effect (McShan and Meyer, 134).

7. *Antithyroid principles.* There are reports of experiments that deal with principles in blood serum, which, following oral administration to animals, inactivate the thyroid and its hormone. Although this particular subject-matter may not be directly within the scope of this review, it deserves brief mention in order to avoid a confusion of these principles with the antihormones. The anti-thyroid principles apparently were discovered in the following manner: The concept of the function of the thyroid gland current in the eighties and nineties of the last century, before the advent of our present knowledge of the physiological actions of the thyroid and its hormone, was that of Schiff and of Horsley, who taught that poisons circulating in the blood were detoxified in the thyroid, and that thyroglobulin represented the iodized, detoxified product. This idea was somewhat modified when the cause of parathyroid tetany was discovered, but a number of investigators continued to believe that a circulating poison is neutralized by the thyroid.

Ballet and Enriquez (10) apparently were the first to attempt to utilize this inhibitory or "toxic" action of the blood for the treatment of Basedow's disease. They fed the serum of thyroidectomized dogs to thyrotoxic patients and claimed beneficial effects. Likewise, Burghart and Blumenthal (21) fed Basedow-patients the serum from myxedema patients, observing equally good results. Möbius (139) had the Merck pharmaceutical house prepare blood from thyroidectomized sheep, called it "Antithyreoidin-Möbius" (140), and he claimed it was beneficial for Basedow's disease. The material was effective only when fed, not when injected. There were many variations of this sort of treatment, including the feeding of milk from thyroidectomized animals.

This concept of antithyroid substances received further elaboration by Blum (16) who called the inhibitory principle "Katechin". He claimed that all normal blood has an antagonistic effect toward thyroid. He showed that the blood of normal or of thyroidectomized sheep prevents in guinea pigs the loss of weight produced by thyroid feeding. Romeis (166a, b) in 1923 showed that "Katechin" prevents the effect

of thyroid on tadpoles; 5 cc. of blood from a thyroidectomized or a normal sheep neutralized 10 gamma of thyroxin in tadpoles. He claimed that red blood cells, serum, and plasma contain "Katechin." Blum (16) stated that these facts were confirmed by Geszner, who found that diiodotyrosine also was neutralized by "Katechin." Although Anselmino and Hoffmann (5) and others found the "Katechin" in an ether-soluble fraction, Blum (16) said the serum still retained its "Katechin" after extraction with ether, that it was water-soluble, heat-stable, and not (or slowly) dialyzable. Blum (16) stated that an ether-extract of blood did not have this effect, and that the effect was not due to cholesterol.

The reports of Anselmino and Hoffmann (5) on "Katechin" are summarized as follows: The active substance was ether-soluble, and was contained in the fatty acid fraction. The following tissues were rich in "Katechin": fetal blood and liver; adult thymus, ovaries, and bone marrow. Normal blood contained less than the above tissues, but the blood in pregnancy and in Graves' disease was deficient in "Katechin." This substance protected the liver from the loss in glycogen caused by thyroid feeding or by the injection of thyrotropic hormone. It caused an increase in the liver glycogen of normal animals and this was not lost during hunger. The substance did not lower the basal metabolic rate of normal animals. Further contributions on this type of anti-thyroid substance have been made by Asimoff (7), by Gürber and Gessner (84), by Lewit, Berland and Rywkin (115), and others (17, 20).

Abelin (2) also has reported on an anti-thyroid substance which may not be the same as the above, but is now mentioned to show the many facets of this problem. From desiccated thyroid tissue Abelin extracted a substance which he said was an iodized peptone, whose active principle was diiodotyrosine. The feeding of this substance to animals caused an amelioration of the symptoms that normally are produced by thyroid feeding. The substance caused the rapid metamorphosis of tadpoles. One half of the number of a group of mice were protected against the toxic action of acetonitrile. When given together with diiodothyroxin it weakened the character of the resultant hyperthyroidism. Lugol's solution did not have any of the above actions in these experiments. Oehme (143) has stated that glycine, which he says has little effect on the metabolic rate, also has an anti-thyroid action. Schäfer (180) has described an antithyroid effect of vitamin C. Another example of the anti-thyroid effect, which calls

to mind the results of Anselmino and Hoffmann, is the finding of Zain (215) that the feeding of unsaturated fatty acids protected rats against the action of thyroid.

Whatever be the nature of the anti-thyroid substances, so briefly mentioned above, they appear on at least two counts to be different from the antihormones, namely: 1, they are active when administered *per os* while the antihormones are not; 2, they probably are not proteins, while the antihormones appear to be globulins.

Antibody formation with thyroid preparations. For the sake of completeness, and for comparison with the data presented on antihormones, some data relative to antibodies for thyroid preparations will be presented. It will be noted that the alluring characteristic of antihormones is their property to inactivate the physiological effects of certain hormones. In this respect the majority of the results with antisera to thyroid preparations differ from those with the antihormones (184). In the review of antihormones by Collip, Selye and Thomson (32a) there is a summary of the formation of antibodies to thyroid preparations that reads as follows:

> . . . The question whether a true immunity with antibody formation may occur as a defence against thyroid extracts was first investigated by Papazolu (1911). This author obtained a positive complement fixation reaction with the serum of patients suffering from Graves' disease against extracts of the thyroids of such patients. Vallagussa (1910) and Kolle (1909) were unable, however, to demonstrate any antibody or antigen in the blood or thyroid of patients suffering from Graves' disease. Ballner *et al.* (1912) found that sera of individuals suffering from goitre, and even sera of non-goitrous individuals, living in the Tyrol where goitre is endemic, give a complement fixation reaction with alcoholic extracts of thyroid tissue. Bauer *et al.* (1937), Bauer (1937), Bauer and Kunewalder (1937) and Bauer and Schächter (1936) found that rabbits chronically treated with thyroxine become resistant to the hormone, and at the same time their serum acquires the property of giving a positive complement fixation reaction with thyroxine or diiodotyrosine. However, they found that this resistance cannot be transferred passively by injecting the serum of a thyroxine-resistant animal into a non-pretreated one. These authors observed, furthermore, that the complement fixation reaction with diiodotyrosine, which is usually positive in cases of severe Graves' disease, became negative following the removal of the hyperactive thyroid. Kestner (1937) reported experiments showing that the serum of sheep chronically injected with pig thyroid emulsions exerts antithyroid effects, in so far as rabbits, dogs and rats treated with this serum show a decrease in metabolic rate. The author believes this to be due to the formation of organ-specific antibodies.
>
> The positive results of immunizing experiments against thyroglobulin (Hektoen *et al.*, 1927; and others) have already been mentioned in the historical introduction and need not be discussed here in detail, since there is little evidence of their having anything to do with antihormones in the true sense of the word, especially

since it was shown that *in vivo* antithyroglobulin fails to antagonize the physiological actions of thyroglobulin (Schulhof, 1930). Rosen and Marine (1937) showed that prolonged injections of iodothyroglobulin do not cause refractoriness to the metabolic action of this substance in the rabbit, in spite of the definite immune response (precipitin formation). It also suffices simply to mention the finding of Picado and Rotter (1936), who obtained specific precipitins against thyroid protein.

THE CHEMICAL PROPERTIES OF ANTIHORMONES. The antihormones appear to be proteins, or are closely linked to them. The active principles reside in the globulin fraction of the serum. This fraction contains the immune bodies, and also certain pituitary and other hormones. The antihormone-containing globulin fraction can be precipitated from other constituents of the blood by methods customary for the preparation of immune globulins. The aqueous solutions slowly lose their activity on standing, but the dried preparations retain antihormone activity for long periods of time; likewise, the aqueous solutions are thermolabile. There is some evidence available to show that in the case of A.P.L. the antihormone-hormone linkage may be broken by simple chemical methods. More exact knowledge of the chemical nature of antihormones awaits studies of more highly purified homogeneous preparations of both the hormones and the antihormones.

The literature is represented by the following: There is general agreement that antihormone activity with respect to pituitary gonadotropic and thyrotropic and to adrenal cortical hormones resides in the globulin fraction of the serum (Harington and Rowlands, 88; Hartman *et al.*, 89b; Thompson, 202a; Zondek *et al.*, 219). Some of the nonspecific antibodies that are not antihormones have been precipitated from the globulin fraction by suitable immunological techniques (Gegerson *et al.*, 73). The desiccated globulin fraction retained its antihormone activity for long periods, and the dried product has been washed with acetone or with ether without significantly altering its potency (Zondek *et al.*, 219, 224). In serum the activity is slowly lost on standing even when sterile and refrigerated, but not when frozen. Collip and Anderson (31a, b) state that the thyrotropic antihormone is completely destroyed by boiling at pH 5 for 3 minutes. The thyrotropic and gonadotropic antihormones apparently do not pass through collodion membranes (Harington and Rowlands, 88). Loeser and Trikojus (126) have described the preparation of thyrotropic antihormone by precipitation with benzoic acid followed by elution of the antihormone from the precipitate.

A number of chemical properties of rabbit A.P.L. antihormone were

investigated by Zondek *et al.*, (221), and the following conclusions were drawn: heating for one hour at 70°C. did not destroy the antihormone activity, which, however, was destroyed by heating to 80°C. and by being brought once to the boiling point in a dilute solution. On the other hand, the acetone-dried powder of an active fraction of antiserum survived dry heating for one hour at 100°C. The antihormone was destroyed by digestion with pepsin, trypsin, and N/10 NaOH. It was not affected by treatment with N/50 NH_4OH, N/10 HCl, 1 per cent hydrogen peroxide solution, nor by ultra-violet irradiation. It was soluble in 40 per cent acetone but not in 50 per cent. It would not dialyze through cellophane or cuprophane membranes. Further experiments led Zondek and Sulman (222) to conclude that the inactivation of A.P.L. by the antiserum was a reversible process, because they apparently were able with exposure to N/10 NaOH to destroy the inhibitory activity in the A.P.L.-antiserum complex, and to release the antihormone from the complex by destruction of the hormone with N/10 HCl. They found the antihormone was contained in the pseudoglobulin fraction of the serum. On the basis of these observations they concluded that the antihormone resembles an immune body, but has certain unusual properties.

Zondek, Sulman and Hochman (225) observed that antihormone neutralizes A.P.L. quantitatively rather than by a process of adsorption, a quality which they say distinguishes this substance from known groups of immune bodies. The same observers elaborated further methods for the preparation of the antihormone to A.P.L., especially studying various methods for its partial purification. They found that the antihormone was precipitated by many of the common protein precipitants, but that the methods of most practical value were precipitations with acetone, or with saturated ammonium sulphate at the isoelectric point of the diluted antiserum. A combination of these methods gave the most highly purified preparations. Antiserum from rabbits appeared to be more suitable for these studies than antiserum from goats.

THE EFFECTS OF ANTIHORMONES IN ANIMALS. Whatever uncertainty there may be in our minds about the chemical and physical nature of the antihormones, there can be no question of the fact that they have physiological actions. This property has called forth the major portion of the experimental data herein reviewed. The physiological effects of antihormones at present are studied by two chief methods, which are: to observe the effects of the refractory state upon the injected animal

itself, and to compare in test-animals these effects with those produced by injections of the serum from the refractory animal. In order to accomplish physiological changes, other than merely to neutralize an injected extract, the antihormone must be either non-species-specific or at least specific for the species of animal used in the test. In order to interpret the changes resulting from treatment with antihormones, some evidence should be submitted to show that the antiserum used was tolerated by the test-animal without ill effects that could be ascribed to anything other than the antihormone. This last condition sometimes has been as difficult to fulfill with antihormone sera as it has been with hormone preparations.

We do not know exactly how the antihormones achieve their effects, but what data we have suggest that the physico-chemical reactions between the hormone and its specific antihormone occur while they both are in the circulating blood stream. The hormone effectively and rapidly is rendered inactive; its later fate is not known. The antihormones to the pituitary hormones have no direct effect on the subsidiary ductless glands, but there is evidence that they may cause some preliminary hyperactivity of the pituitary itself, and this may, if the action is prolonged, lead to exhaustion atrophic effects in this gland. The effect of the antihormone, then, in addition to neutralizing a coincidentally injected extract, is also to inactivate hormones while they are *en route* in the animal itself from pituitary to subsidiary ductless glands. This creates with respect to the antihormones injected a sort of therapeutic selective hypophysectomy. An atrophy of the appropriate subsidiary glands subsequently occurs, as if hypophysectomy had been performed, and the final physiological effects observed are the results of these atrophies. The more detailed account follows:

Effects of gonadotropic antihormone. Rowlands (169, 170b) together with his associates (150, 175a, b, 177) have reported possibly the most complete experiments on the actions of antihormones. A non-species-specific antihormone in rabbit serum had the following effects: 1, inhibited ovulation and corpus luteum formation in the donor rabbits; 2, caused atrophy of the reproductive organs of the adult male rat, and this occurred in the same time-interval that it does after castration or hypophysectomy; 3, suppressed ovulation in the pubertal rat; 4, inhibited implantation of the blastocyst in the rabbit, or caused resorption of the fetuses if injections were begun late in pregnancy. This serum had no effect on the lactation of rats. The foregoing experiments and those of Thompson and Cushing (203b) and Thompson (202c) supple-

ment each other. In these latter experiments a non-species-specific canine antihormone had the following effects: 1, the development of the reproductive system of young rats was held in abeyance during the course of the injections; 2, the pregnancies of dogs were terminated within 100 hours after the first injection, without any evident directly harmful effects on the fetuses.

In their first reports, Collip and his associates (9, 188a, d, e), had shown there was an atrophy of the gonads of the refractory animals; in some cases there were "wheel cells" present in the theca (32c), a finding which is said to be a sign of complete absence of hypophyseal gonadotropic stimulation (188b, c). Failures (226) (and there have been some) to achieve physiological effects in such situations as the foregoing probably have been due to a lack of specificity of the antihormone in the test-animal.

On the anterior pituitary gland the pituitary antihormones have pronounced functional (64, 105) and histopathological effects, and for the convenience of the reader all of the data on this subject will now be given, although some data have to do also with thyrotropic and adrenotropic and possibly other antihormones. The first of the histopathological data comes from the early work of Koyano (104), 1923, whose cytological description of pituitaries affected by an antihormone preparation is remarkably like the later and more complete one of Severinghaus and Thompson (189a, b), who found that the pituitary glands of long-time refractory dogs had all the changes characteristic of castration, of thyroidectomy and possibly of adrenalectomy.

Although numerous reports had been made of "signet-ring" or "castration changes" having been produced in the anterior lobe basophils by antihormones, it appears that Severinghaus (189a, b), the cytologist, was the first to coördinate the observed changes with the new knowledge of pituitary cytology in endocrine disorders. In this particular investigation, the following 3 types of pituitaries were observed: the first were from 2 sheep that had long been injected with a sheep pituitary extract that did not call forth antihormones; the second were from 2 dogs that had developed non-species-specific antihormones after prolonged injections of the sheep pituitary extract; and the third were from 2 dogs injected for 30 days with canine antihormone serum similar to that which might have been obtained from dogs of the second group. Briefly stated, the results showed that the pituitaries of the sheep (without antihormones) showed the characteristic effects of estrogenic hormones, due undoubtedly to the active ovaries of the animals; the

pituitaries of the refractory dogs showed advanced mixed effects, some basophil cells being characteristic of thyroidectomy, and others of castration, while at the same time there was degranulation of the acidophils; the basophils in the pituitaries of the antihormone-injected dogs showed the changes described by Crooke. This change, a characteristic hyalinization of the cytoplasm of the basophils, is found in practically every case within the Cushing syndrome (51).

Effects of thyrotropic antihormone. Anderson and Collip (3) observed that this antihormone causes the basal metabolic rate of rats to decrease below the normal level, and the effect of thyrotropic hormone on the metabolic rate to be inhibited. Before the phase of refractoriness actually is apparent the liver regains its glycogen content which had disappeared following the original action of the thyrotropic hormone (Fischbach and Terbrüggen, 66). Loeser (122) has shown that the thyrotropic antihormone prevents the liver-glycogen depleting effect of thyrotropic hormone. The normal thyroid gland of the guinea pig is said to become atrophic after treatment with the antihormone (54). Anderson and Collip (3), and Eitel and Loeser (55) have found that the thyrotropic hormone content of the hypophysis is reduced following treatment with the antihormone.

Other antihormone effects. The actions of antihormones to prolactin, to corticosterone, and to the diabetogenic hormone have been described in earlier sections to which the reader is referred. Certain unusual antagonistic effects ascribed to antihormones might be mentioned, such as the following: Russell (178) has reported on the production of refractoriness to the action of anterior pituitary extracts in depressing oxidation of fed carbohydrate. Rodewald (165a, b) claims that in serum from patients suffering with cancer there may be an antihormone having the property to neutralize the action of A.P.L., and also another which inactivates the melanophore principle. Durupt *et al.* (45) have reported on the presence in the urine from certain women of a substance which are antagonistic to estrin. There is not sufficient data presented to indicate the nature of the inhibitory effect. Also reported (183) is a property of serum of pregnant women said to inactivate vasopressin in test-animals.

Here it may be of interest to note that all efforts by the injection of pituitary extracts to reproduce the Cushing syndrome have failed because refractoriness to the adrenotropic hormone has developed in the animals. The adrenotropic factor is believed by some to be overproduced in patients with basophilism, especially in the cases with

basophilic adenomas and also in the cases where there is no tumor of any gland.

An interesting animal prepared by Thompson and Cushing (203a) perhaps deserves description. Unusual anatomical changes were produced in a fox terrier puppy that was injected for four months with large doses of a crude, ovine pituitary extract. The animal became adipose; had a strikingly sub-normal metabolic rate as compared to the control; had a retardation in the osseous development with incarceration of the teeth and the development of prognathism; the liver became tremendously enlarged, with glycogen deposits of an extent seen only in von Giercke's disease; the skull was eburnated and had the changes which usually follow hypophysectomy. In some respects the effects of the injections were similar to the findings in basophilism, but in others they resembled hypophysectomy, for the subsidiary ductless glands were strikingly atrophic.

THE SIGNIFICANCE OF ANTIHORMONES. The antihormones are an important class of substances because of their physiological properties which induce the refractory state and alter certain functions of the ductless glands. Their discovery added to our knowledge by introducing a new concept and by further revealing the breadth of an animal's protective reactions to unusual conditions. When they were first described the importance of antihormones was recognized by physicians (47, 48) who appreciated not only the possible dangers which might result in patients from the induction of the refractory state but also the value should their actions receive successful therapeutic application in certain endocrine disorders.

Most of the hormones now available for therapeutic use must be administered parenterally. The induction of the refractory state in a patient obviously must be avoided by using for the therapy non-antigenic preparations. These are homologous preparations, or those of sufficient purity that they have no antihormone-producing power. The pharmaceutical houses should without delay seek methods to remove the antigenic qualities of their endocrine products intended for parenteral use.

The successful therapeutic use of antihormones in man has yet to be reported. A number of difficulties have stood in the way of those who have attempted to test their actions in man. The toxic factors present in many animal sera must be removed before the preparation may be tested (100). The antihormone must be specific not only for the hormone but also for the species, or it must be completely non-

species-specific, able to accomplish the same effect as a specific antihormone.

It is not easy to prepare large quantities of a suitable specific serum, for when the antihormone potency is sufficiently great the non-species-specific effects on the injected animal are such that bleeding in any quantity is not well tolerated, and the animal also becomes susceptible to other ailments. Despite these difficulties, which may be overcome, there is need for tests of the actions of antihormones to be made, with suitable precautions for safety, in man. Valuable physiological data will be obtained no matter what may be the physiological action as judged from the therapeutic standpoint. One cannot predict the effects to be observed in man following the administration of properly prepared specific antihormones, for knowledge of the hormonal balance in hyperthyroidism, in pregnancy, and in other states in which antihormones might be tested is still incomplete. Conceivably the endocrinologist may utilize the action of specific antihormones in the study of the interrelations of the endocrine glands.

The study of antihormones is founded on a broad base, and like so many problems of the present this requires the application of a number of techniques. There is great need for well planned investigations of antihormones by the immunologists, who should have the close collaboration of endocrinologists, for the serological and biological tests must be parallel. What the investigators need for this study are highly purified and homogeneous preparations of the hormones and antihormones.

In conclusion one may state that the antihormones now appear to be immune bodies of unusual type. These substances have important properties whereby they inactivate hormones and thus indirectly produce changes in the ductless gland physiology. Although in some circumstances they are unwanted "artifacts" or complicating factors, their physiological actions conceivably may find some useful application.

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CONTENTS

	PAGE
FACTORS AFFECTING THE TESTS OF KIDNEY FUNCTION. *Raymond C. Herrin*	529
ANAPHYLAXIS. *Carl A. Dragstedt*	563
ANTIHORMONES. *Kenneth Wade Thompson*	588

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Contents of Volume 21, 1941

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BERTA SCHARRER: Endocrines in Invertebrates

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JACOB SACKS: Changing Concepts of the Chemistry of Muscular Contraction

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R. C. HERRIN: Factors Affecting the Tests of Kidney Function

O. H. ROBERTSON: Phagocytosis of Foreign Material in the Lung

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K. A. C. ELLIOTT: Intermediary Metabolites and Respiratory Catalysis

I. GERSH: Recent Developments in Histochemistry

SAMUEL SOSKIN: The Blood Sugar: Its Origin, Regulation and Utilization

INDEX

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Contents of Volume 20, 1940

VICTOR C. MYERS AND EDWARD MUNTWYLER: Chemical Changes in the Blood and Their Clinical Significance

M. O. SCHULTZE: Metallic Elements and Blood Formation

CLAY G. HUFF: Immunity in Invertebrates

PAUL R. CANNON: The Functional Significance of Specific Agglutinins and Precipitins

CARL F. SCHMIDT AND JULIUS H. COMROE, JR.: Functions of the Carotid and Aortic Bodies

ALFRED BLALOCK: Experimental Hypertension

SIDNEY C. MADDEN AND GEORGE H. WHIPPLE: Plasma Proteins: Their Source, Production and Utilization

RUDOLF SCHOENHEIMER AND D. RITTENBERG: The Study of Intermediary Metabolism of Animals with the Aid of Isotopes

C. A. ELVEHJEM: Relation of Nicotinic Acid to Pellagra

WALTER BAUER, MARIAN W. ROPES AND HANS WAINE: The Physiology of Articular Structures

ESTHER M. KILLICK: Carbon Monoxide Anoxemia

WILLIAM T. SALTER: Fluctuations in Body Iodine

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KONRAD DOBRINER AND C. P. RHOADS: The Porphyrins in Health and Disease

WILLIAM H. TALIAFERRO: The Mechanism of Acquired Immunity in Infections with Parasitic Worms

H. G. SWANN: The Pituitary-Adrenocortical Relationship

MILAN A. LOGAN: Recent Advances in the Chemistry of Calcification

A. C. FRAZER: Fat Absorption and Its Relationship to Fat Metabolism

D. A. GREENWOOD: Fluoride Intoxication

Tentative Contents of Volume 22, 1942

GEORGE WALD: Carotinoids and Vitamins A in Animal Metabolism

CHARLES L. YUILE: Hemoglobinuria

CARL J. WIGGERS: Present Status of the "Shock" Problem

S. B. WOLBACH AND OTTO BESSEY: Vitamins and Pathological Tissue Changes

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D. W. BRONK: Unitary Analysis of Reflex Activity

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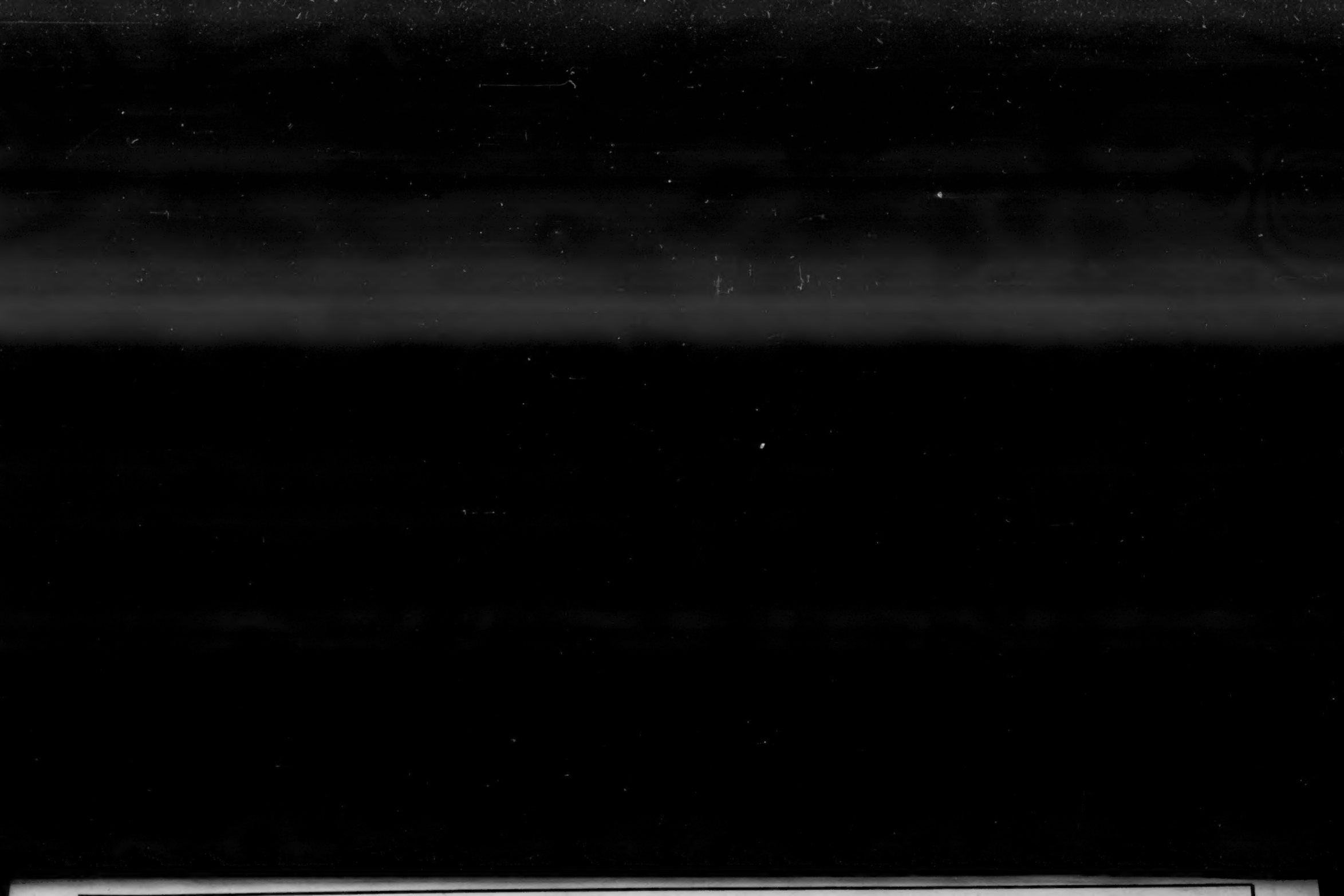
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